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13. ABSTRACT ***(Maximum 200 Words)***

Sentinel lymph node biopsy (SLNB) was evaluated in 535 breast cancer patients in a multi-center trial using a combination of isosulfan blue and technetium sulfur colloid to *1)* determine if the SLN accurately predicts the disease in the remaining axilla; *2)* identify factors that affect the success and false negative rate; *3)* identify molecular markers to assess the prognostic value of detecting SLN micrometastatic disease using RT-PCR. SLNB by experienced surgeons can detect the SLN in >90% patients, with a false negative rate<5%. Only patient age >50 years and surgical experience<30 cases increased failure and false negative rates. Prior surgery, tumor size/location, vascular/lymphatic invasion, or method of diagnosis had no effect. The optimal timing of Tc99 injection (1-5 hrs) and injection site (intradermal and combination of sites appear superior) were investigated. Analysis of 154 SLN from 87 patients indicates that mammaglobin and CEA, alone or in combination, are highly specific (undetectable or trace levels in normal LN), highly accurate (detect tumor cells in 84-97% breast cancer patients) and highly sensitive (detect occult disease and potentially upstage 25-44% histology-negative patients) markers. There was a 73% concordance rate in mammaglobin and CEA expression. Marker expression correlated with tumor size and ER status.

14. SUBJECT TERMS
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INTRODUCTION

Sentinel Lymph Node Biopsy (SLNB) is a minimally invasive surgical method that identifies the lymph node(s) (sentinel lymph nodes) that primarily drain the tumor and thus are most likely to harbor metastatic disease. The detection of disease in axillary lymph nodes (LN) is the single most important prognostic indicator in patients diagnosed with breast cancer and helps guide the selection of treatment modality.

The first goal of the proposed studies is to determine if the SLN accurately predicts the presence of disease in the remaining axillary LN in breast cancer patients, using a defined protocol in a multi-center trial. We aimed to define the population of women who can benefit from this procedure and to determine factors that contribute to the success and failure of this procedure. The significance of this work is that patients that truly lack metastatic disease can potentially avoid the unnecessary significant medical and surgical risks of complete axillary LN dissection.

The second goal of the proposed studies is to determine if Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) analysis of SLN for specific markers of micrometastatic disease will result in improved sensitivity over routine histological methods. After surgical removal, one section of each lymph node has been typically examined by pathologists for the presence of metastatic disease. This method fails to identify a large number of breast cancer patients (18-30%) with metastatic disease. SLNB allows a more focused and comprehensive analysis of the 1 or 2 SLN for the presence of metastatic disease and also enables us to evaluate RT-PCR as a more sensitive method. The significance of this work is that patients that truly lack metastatic disease can potentially avoid the unnecessary morbidity associated with aggressive therapy and that those patients histology-negative nodes that actually have metastatic disease will be identified and treated appropriately.

BODY

Our research group has completed the approved tasks (Technical Objectives 1-5) outlined in the Statement of Work for the second year of grant funding. We will discuss the clinical / surgical achievements and the laboratory / PCR achievements separately under each Technical Objective.

A. Clinical multi-institutional study to investigate the accuracy and significance of SLNB in the management of breast cancer.

Technical Objective 1. "Complete SLNB on 500 breast cancer patients"
Technical Objective 3. "Determine accuracy of SLNB in predicting the status of the remaining nodes by reviewing the data accumulated on patients in the first two years"
Technical Objective 4. "Determine success rate of SLNB to find the sentinel node by reviewing the data accumulated on patients in the first three years"

Surgically led by Dr. Lorraine Tafra, we were among the first centers in the world to implement SLNB in breast cancer patients and to begin a multi-center trial to evaluate this surgical technique. In 1996, we initiated a clinical trial with breast cancer patients to determine if the SLN could predict the presence of disease in the remaining draining LN, as it does with melanoma. This required performing SLNB followed by full node dissection in all patients to allow an accurate calculation of the false negative rate, i.e. to determine the number of patients with histology negative SLN(s) but histology-positive non-sentinel LN(s). The trial was expanded to a multi- center trial early in 1997. The centers were enrolled after attending the ECU or Anne Arundel CME course "Lymphatic Mapping and Sentinel Node Biopsy: Indications, Techniques, and Prospects in the Management of Malignancies" in which Dr. Tafra served as course director, inconjunction with five other nationally known faculty. In addition to interactive instruction on all aspects of sentinel node biopsy, this course included hands-on training in a porcine laboratory model. Dr. Tafra has visited nearly every site to provide direct surgical training in the SLN biopsy procedure. We have also assisted the centers with obtaining IRB approval, and with pathology and specimen handling guidelines. Half of each SLN, half of one non-sentinel LN and a portion of tumor (when available) were frozen and banked for PCR analysis.

A detailed description of our analysis of 535 patients can be found in the appended manuscript, which has been accepted for publication to the Annals of Surgery[1](enclosed in the Appendix). This is the report of the first multi-center trial to use a combination technique of isosulfan blue dye and technetium sulfur colloid to locate the sentinel node in breast cancer patients. These data support the first hypothesis of our research proposal that SLNB, using a combination of isosulfan blue, Tc99 and the intra-operative gamma probe, can detect the sentinel node in >90% of patients with a small false negative rate. This work was presented at the 22nd Annual San Antonio Breast Cancer Symposium in December 2000[2] and at the Dept. of Defense Breast Cancer Research Program ERA OF HOPE meeting in June 2000[3] (abstracts enclosed in the Appendix).

Of 535 patients the overall success rate in finding a SN (identification rate) was 88% and the false negative rate was 13%. The accuracy of the SLN to detect metastatic disease was 96% and the negative predictive value was 95%. Patient age (50 years and older) and surgeon experience were the primary factors contributing to the failure to locate a SLN. The success rate increased, and the false negative rate decreased to 90% and 4.3% respectively, after surgeons had performed more than 30 cases. In elucidating surgical experience as a critical factor for successful SLNB, our trial was instrumental for the American Society of Breast Surgeons and the American College of Surgeons consensus statement, co-authored by Dr. Tafra. The initial consensus was that SLNB for breast cancer patients has become standard of care in the hands of experienced surgeons that are enrolled in a clinical trial and have performed at least 30 operations with a false negative rate <5%[4]. In the 2000 iteration of the consensus statement[5] (enclosed in Appendix), the minimum number of cases has been decreased to 20 cases. The surgeon credentialing issues that have arisen are discussed in some detail in Reference 6 and a general overview of the current state of sentinel lymph node biopsy is reviewed in Reference 7 (See preprints in the Appendix).

In addition to emphasizing the importance of surgical experience, data from the trial have impacted the surgical community by showing **1)** the usefulness of a combined injection technique of Tc99 and blue dye for SLN localization; **2)** the application to larger tumors and patients with prior open biopsy or lumpectomy; **3)** that removal of all SLNs, not solely the most radioactive, is needed for a low false negative rate [1,8-12].

This multi-center trial, from both private practice and academic institutions, is an excellent indicator of the general utility of SNB. It establishes those factors that play an important role in failure and false negative rates (patient age, surgical experience, tumor location) as well as those that are irrelevant (prior surgery, tumor size, Tc99 timing). This widens the applicability of the technique as well as identifying factors that require further investigation.

We have addressed additional clinical questions this past year by re-analysis of the data. These studies have been presented or have been accepted for presentation at national meetings, are included in the Appendix and will be summarized in the following section.

There is a lot of controversy surrounded the issue of tumor size and performance of sentinel node biopsy. Although it has been clearly established that the chance of harboring metastatic disease increases as the size of the tumor increases, it is very difficult to predict who and who will not have metastatic disease. The only way to test whether the technique was useful in patients with larger tumors was to perform sentinel node biopsy accompanied by a completion axillary node dissection. Our data revealed that although a larger number of patients with larger tumors (>4cm) do have metastatic disease there are patients who can be spared an axillary node dissection because they do not have metastatic disease[14]. Of equal importance was that the false negative rate and the identification rate did not differ from those patients with smaller tumors treated with sentinel node biopsy. Providing this data now enables patients with good prognostic tumors that are larger to be treated with sentinel node biopsy alone and reserve completion axillary node dissection for only those patients with positive sentinel nodes.

Prior to presentation of our study[15], it remained unclear what Tc99 time interval (time interval from injection of the Tc99 to dissection of the sentinel node) resulted in the highest identification rate and the lowest false negative rate. Most studies have restricted the timing but in an effort to evaluate this factor in sentinel node biopsy our investigators were allowed to choose their injection time intervals. It seems reasonable that the longer the injection interval the higher the success rate, as the sentinel node is allowed to concentrate the Tc99 in the sentinel node. This higher concentration of the Tc99 allows the

sentinel node to be distinguished at the level of the skin, prior to the skin injection, using the gamma probe. However, as the half life of Tc99 is 6 hours, the interval can not be too long or the Tc99 will not be detected at all. The data from our clinical trial revealed an optimal interval between 1-2 hours with a very high identification rate and low false negative rate. Ours is the larger series reported with data on timing interval.

There are different methods of injection used for lymphatic mapping and sentinel node biopsy. One study found a very high identification rate when the Tc99 was injected into the skin (intradermally, the method used for melanoma) compare to subcutaneously. We first initiated a study in an animal model to determine if there were actually transit time differences when lymphatic mapping agents were injected into these two sites[13]. It was clearly established that the transit time for most of the mapping agents is much slower in the subcutaneous site compared to the intradermal site and these differences in the pig model were statistically significant. The work has been submitted to the Journal of Surgical Research.

Another injection technique has been reported using Sappy's plexus injection. The advantage of this site is that it takes the radioactivity away from the upper outer quadrant near the sentinel nodes and places it more centrally. This makes it easier for the sentinel node to be isolated from the primary tumor which can be especially difficult in patients with upper outer quadrant lesions. The false negative rate and identification rates however are not well documented with this technique. In order to compare this technique with the established technique of intradermal and subcutaneous (or peri-tumor) injection technique a subset of patients underwent mapping employing all techniques with differential agents[16]. Intradermal injection over the tumor did identify the largest number of SLN ($n=94$; $p<0.001$) when compared to intraparenchymal peri-tumor injection ($n=82$), or subcutaneous injection in Sappy's plexus ($n=72$). In addition, intradermal injection identified the greatest number (17/19; 90%) of the histological-positive SLN, followed by Sappy's plexus (17/19; 84%) and peri-tumor (14/19; 74%) injections, however these numbers are too small to attain statistical significance. Thus, these data indicate that intradermal injection should be used and suggests that all three techniques combined may yield the greatest sensitivity.

Future clinical work:

The results of the clinical trial have raised a number of important issues that remain to be resolved if the technique of SLNB is to be widely accepted and applicable to the majority of breast cancer patients. Some of these questions will require the design of other clinical trials. Our current trial continue to yield data that will determine the significance of immunohistochemical positive patients as well as the usefulness of sentinel node biopsy in patients with DCIS. All of the data will need to be reanalyzed eliminating the "training" or initial 20-30 cases performed by the investigators to determine if there remain any other factors that influence the false negative and identification rate. The sentinel node technique is clearly more accurate in staging breast cancer patients and data analysis of the patients in this series compared to patients not undergoing sentinel node biopsy may reveal a group of patients with a much better prognosis with sentinel node negative disease. It may reveal a group of patients that truly do not need any form of adjuvant therapy. Some questions can also potentially be answered after accrual of additional patients (we are still accruing patients in our trial and have increased our final target number to 750 patients). The most important question that remains is what factors contribute to the false negative rate. We learned in our first 500 patients that even with this large group of patients, analysis is difficult secondary to the small number of patients that actually have a false negative result. A larger group of patients will provide more patients with a false negatives result and allow more accurate data analysis.

B. Laboratory PCR study to investigate the accuracy and significance of PCR detection of SLN micrometastases in breast cancer patients.

Technical Objective 2: Determine the markers needed for the PCR panel.

As reported last year, before prematurely performing PCR analyses on patient samples, we

concentrated on defining the optimal marker(s). We have published our initial study to develop a panel of markers for RT-PCR detection of breast cancer micrometastases in SLN[17,18]. A detailed description of our study, including tables and figures, can be found in our *Cancer Research* article[18] and in last year's progress report.

We were the first to report the use of mammaglobin as a marker for the detection of SLN metastases. Our group has played a major role in recognizing mammaglobin's potential for this application and in communicating our laboratory results to the scientific community both in publications[18] and in meeting presentations[17,19-21](recent abstracts enclosed in the Appendix). Based on our initial oral presentation at the 1998 AACR meeting and on the subsequent Cancer Research article, many investigators in the field have abandoned K19 as a nonspecific marker and have begun to investigate mammaglobin and CEA[22-25]. Indeed, based on our work, three other investigators have recently reported mammaglobin and/or CEA mRNA expression in LN of breast cancer patients by in situ hybridization or RT-PCR[22-25].

The background data on these markers have been discussed in much detail in the grant proposal and in last year's report and will only be summarized briefly here. Mammaglobin is a breast-specific marker that is frequently overexpressed in breast tumors. Mammaglobin expression is completely absent from all other tissue tested, including peripheral leukocytes, lymph node, and bone marrow. Mammaglobin is a member of the uteroglobin family, which includes lipophilin a and b. Although it's function remains to be elucidated, recent data indicates that native mammaglobin exists as a heterodimer with lipophilin b, with which it shares ~35% homology (Tim Fleming, personal communication).

CEA, the second marker that passed our stringent RT-PCR screen, is a notable tumor marker for gastric and colorectal cancer that is also expressed by the majority of breast tumors. Although unlikely to be a good single marker, CEA is an excellent candidate for a multi-marker panel. CEA has been used as an RT-PCR marker to detect carcinoma cells in lymph nodes, bone marrow and blood of breast cancer patients. PCR detection of CEA-positive colon lymph node metastases is a significant prognostic factor in patient survival.

Technical Objective 5. Analyze patient tumor, sentinel node and non-sentinel node specimens by PCR.

We have completed analysis of 154 LN from 87 patients enrolled in our trial, for both mammaglobin and CEA expression. Preliminary results were reported at the 23rd Annual San Antonio Breast Cancer Symposium in December 1999[20], the DOD Era of Hope meeting in June 2000[3] and these data will be presented fully at the 24th Annual San Antonio Breast Cancer Symposium in December 2000[21] (reprints enclosed).

Patient specimens were un-blinded only after PCR analysis and before data analysis for presentation. In this initial patient cohort, an average of 1.8 SLN were removed from each patient. By histologic analysis, 37% patients were node-positive and 63% node-negative, consistent with general clinical observation. A representative RT-PCR analysis of breast cancer patient specimens for mammaglobin expression is shown in ***Fig 1*** (page 6). Last year we formed a research collaboration with the National Genetics Institute (NGI) and worked closely with NGI to transfer the CEA nested PCR protocol to their automated system using Southern Blot analysis. ***Fig 2*** is a representative Southern Blot analysis of breast cancer patient specimens for CEA expression.

The use of CEA as a molecular marker is somewhat controversial, as others have reported it expressed in non-tumor tissue. In our hands using nested PCR at ECU, we did not detect CEA transcripts in 20 normal nodes[17,18]. Ooka and colleagues also failed to see CEA in normal LN controls[25]. When subjected to the more sensitive Southern blot assay, we did begin to see false positives. We evaluated two sensitivity levels for this assay, using 30 or 32 cycles of amplification. Thirty cycles was slightly more specific, but we could still detect CEA in some negative controls. We decided to use 32 cycles for our analysis because of the slight sensitivity advantage.

Two "normal" control LN produced consistently high signals often greater than the tested SLN at either sensitivity level. Interestingly, these two normal nodes turned out to be cervical nodes from the same male undergoing carotid endarterectomy. This individual was found to have developed basal cell carcinoma on his nose four months after the surgery and lymph node procurement. There are isolated

reports in the literature of CEA expression by basal cell carcinomas, so it is possible that these LN were, in fact, not "normal". Based on these findings, all LNs from this patient were removed from our analyses.

Nevertheless, there is a low-level "background" expression noise observed with CEA in normal LN tissue using Southern Blot analysis. To ensure a representative background signal was obtained, 25% of the samples of a given PCR run were normal nodes. Blots were scanned and quantitated and the average and peak normal node values were compared to SLN values. A positive signal was defined as 1.5 times the background. In each PCR reaction batch, each node was analyzed in quadruplicate representing two different cDNA preparations (normal nodes were run in duplicate from one cDNA). For an individual node to be positive, at least half of the replicates had to be greater than 1.5X the peak and mean NLN values.

A summary of the PCR results of SLN from histology-positive patients is shown in **Table 1** (page 6). An average of 1.8 SLN were identified per patient in this cohort. When these samples were analyzed for mammaglobin using our standard RT-PCR conditions, 91% (41/45) histology-positive SLN were PCR-positive, reflecting mammaglobin expression in 94% (30/32) node-positive BrCa patients. CEA expression was detected in 82% (37/45) histology-positive SLN, reflecting CEA expression in 84% (27/32) node-positive BrCa patients. These results are consistent with our in vitro data and with indications from the literature. Only one patient with histological-positive SLN failed to express either marker (false negative rate = 3%; 1/32 patients). These data indicate that PCR analysis for these markers is highly accurate - it detects tumor cells in SLN in the vast majority of histology-positive breast cancer patients.

A summary of the PCR results of SLN from histology-negative patients in shown in **Table 2.** Mammaglobin was expressed in 24% (26/109) of the histology-negative SLN by RT-PCR, thus increasing the detection of occult tumor cells and potentially upstaging 27% histology-negative patients (15/55). CEA was expressed in 23% (25/109) of the histology-negative SLN by RT-PCR, thus increasing the detection of occult tumor cells and potentially upstaging 25% histology-negative patients (14/55). Thus, PCR analysis for these markers is highly sensitive. Each marker increased the detection of occult metastases; PCR upstaged 24-44% histological-negative breast cancer patients, depending on the marker or combination of markers used.

There was a 73% concordance rate in the expression of mammaglobin and CEA in SLN. It was somewhat unexpected that only 9% of the histology-negative SLN (10/109) and patients (5/55) expressed both markers. It is unclear if this selective expression will be maintained when larger numbers of samples are accrued. It was unanticipated that micrometastatic cells would tend to express only one of these markers, when the majority of tumors and histology-positive LN tend to express both markers.

Marker expression was positively correlated with tumor size >5 cm ($p>0.05$) and estrogen receptor negativity ($p=0.01$). The first 92 patients enrolled and analyzed by RT-PCR are currently at a mean 3 years post-surgery. We have lost 5 patients due to noncompliance with follow-up. Only 5 patients have recurred in this group of patients to date, all of whom had histology-positive SLN, thus the numbers are still too small to analyze for the prognostic value of PCR.

Future Laboratory Work

This year we have focused our efforts on analysis using mammaglobin and CEA because the initial in vitro data appeared so promising. This next year we will also evaluate new primer candidates for the panel. As before, control LN (acquired from non-cancer patients as by-products of several surgical procedures) will be tested for expression of other potential markers by RT-PCR. Only those markers that ***1)*** are expressed in most human BrCA lines and ***2)*** are absent from all 20 normal LN will be added to the panel for evaluation of specimens from patients enrolled in the SLNB trial. Several of the promising new candidates include urokinase plasminogen activator (uPA) and Plasminogen Activator Inhibitor-1 (PAI-1) (markers of tissue invasion and metastasis); the prolactin-inducible protein (PIP) and Prostate Specific Antigen (PSA).

We will be continuing on Technical Objective 6: To Determine the significance of a PCR-positive, histologically negative SLN. Clearly, PCR for mammaglobin and CEA is a specific, sensitive method to detect breast SLN micrometastases. We have found that the accuracy of these markers alone is

Figure 1: Representative RT-PCR Analysis of Breast Cancer Patient Specimens for MammaglobinExpression.

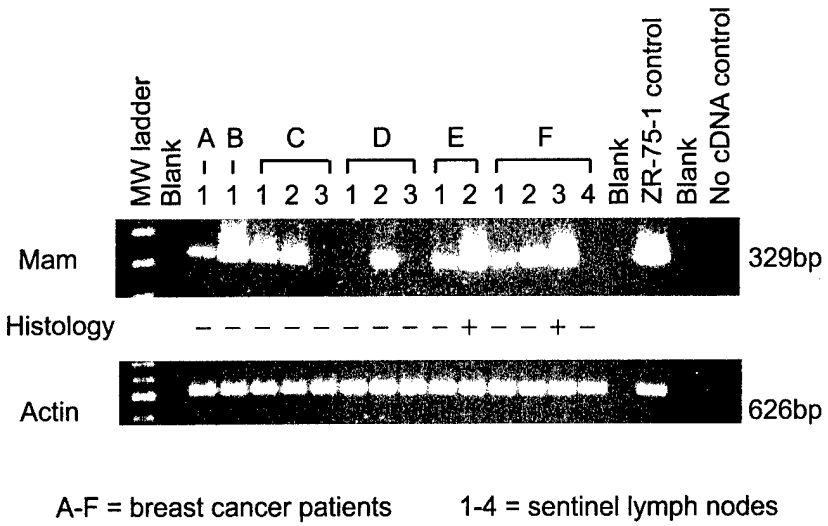

Figure 2: Representative RT-PCR Analysis of Breast Cancer Patient Specimens for CEA Expression.

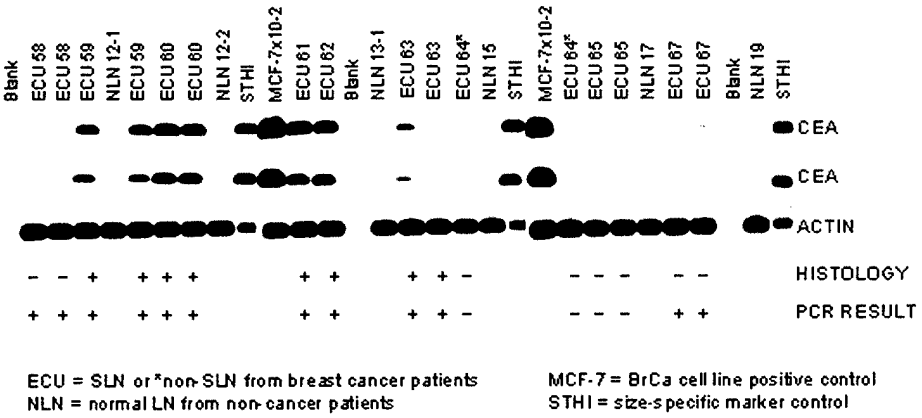

Table 1: Marker Expression in Histologically Positive Specimens.

	SLN	PATIENTS‡
MAM POSITIVE	41/45 (91%)	30/32 (94%)
CEA POSITIVE	37/45 (82%)	27/32 (84%)
MAM AND CEA POSITIVE	34/45 (76%)	25/32 (78%)
MAM OR CEA POSITIVE	44/45 (98%)	31/32 (97%)

‡ Based on the finding of at least one PCR positive SLN in a given patient (average # SLN/ patient = 1.8 in this cohort)

- Only one patient with histologically positive lymph nodes failed to express either marker (false negative rate = 3%).

Table 2: Marker Expression in Histologically Negative Specimens.

	SLN	PATIENTS‡
MAM POSITIVE	26/109 (24%)	15/55 (27%)
CEA POSITIVE	25/109 (23%)	14/55 (25%)
MAM AND CEA POSITIVE	10/109 (9%)	5/55 (9%)
MAM OR CEA POSITIVE	41/109 (38%)	24/55 (44%)

‡ Based on the finding of at least one PCR positive SLN in a given patient (average # SLN/ patient = 1.8 in this cohort)

- Mammaglobin and CEA detect occult disease and may upstage 25-44% of histologically negative patients.

extremely high, as detailed above. Long term follow-up for recurrence will be required to determine which molecular detection level is clinically significant. It will be important to determine with larger numbers of specimens if CEA and mammaglobin have complementary expression in tumor and LN and if both markers combined are superior to one marker alone for both detection and prognostic value. Four year follow-up data in this initial patient cohort will be available by year 3 of this grant.

Key Study Questions we will be asking in our statistical analysis of the followup data include:

1) Is the detection of micrometastatic disease in SLN prognostic of disease recurrence and survival?
2) Are there any differences between PCR and histology in their ability to detect micrometastatic disease in SLN and predict disease recurrence and survival?
3) Are there optimum PCR markers for detecting micrometastatic disease in SLN?
4) Is there an optimum level of PCR detection sensitivity for detection of SLN micrometastases and prediction of disease recurrence and survival?

RESEARCH ACCOMPLISHMENTS

- Validated the technique of SLNB ability to predict the status of the axillary nodes in academic and non-academic settings using a combination technique of Tc99 and isosulfan blue
- Established the learning curve required before a high identification rate is obtained
- Established those factors that significantly influence the results of SLNB: patient age > 50 and surgical experience > 30 procedures.
- Determined those factors that were previously thought to influence results but found to be irrelevant: including prior surgery on the breast (open biopsy) and larger tumors. This widens the applicability of the technique to patients that would previously have been excluded.
- Determined that the optimal time interval between Tc99 sulfur colloid injection and SLNB is between 1 and 5 hrs.
- Determined that intradermal injection over the tumor identified the greatest number of SLN and that the use of a combination of injection sites may be beneficial.
- Confirmed that mammaglobin and CEA as highly specific RT-PCR markers for breast cancer detection in SLN (i.e. transcripts are undetectable or present at very low levels in normal LN).
- Determined that these markers are highly accurate (i.e. detect tumor cells in 82-98% histology-positive SLN and 84-97% breast cancer patients, depending on the marker and combination of markers).
- Determined that these markers are highly sensitive, can detect occult disease in histology-negative SLN and may potentially upstage 25-44% histology-negative breast cancer patients.

REPORTABLE OUTCOMES (9/99-10/00)

A. **Manuscripts, Abstracts, Presentations**

Manuscripts in press:

1. **Tafra, L**, Lannin, D, Swanson, MS, VanEyk, J, **Verbanac, KM**, et al. "Findings of the first multicenter trial of sentinel node biopsy for breast cancer using both technetium sulfur colloid and isosulfan blue dye", *Annals Surg.*, 2001.

2. **Tafra, L.**, McMasters, K., Whitworth, P., Edwards,M. Credentialing Issues with Sentinel Lymph Node Staging for Breast Cancer. American Journal of Surgery. In press.

3. **Tafra, L.** State of Affairs of Sentinel Node Biopsy for Breast Cancer. *Current Surgery*, 58(3), 2001.

4. Edwards M, Giuliano A, Reintgen D, **Tafra L.** Revised Consensus Statement on Guidelines for Performance of Sentinel Lymph Node Biopsy for Breast Cancer. *American Society of Breast*

Surgeons: Quarterly; Fall 2000.

Abstracts (Included in Appendix):

1. **Verbanac, KM**, Fleming, T, Min, CJ, Purser, S, **Tafra, L.** (1999) "RT-PCR increases detection of breast cancer sentinel lymph node micrometastases", 22nd Annual San Antonio Breast Cancer Symposium, *Breast Cancer Research and Treatment 57:41 #125.*

2. Ng, PC, Chua, AC, Lannin, DP, VanEyk, JJ, Swanson, MS, **Tafra, L.** (1999) "Age and surgeon experience: the only significant factors contributing to sentinel node mapping failure in breast cancer", 22nd Annual San Antonio Breast Cancer Symposium, *Breast Cancer Research and Treatment.* 57:27 #12.

3. Kersey, T, VanEyk J, Lannin D, **Tafra L** (1999) "Comparison of Intradermal and Subcutaneous Injections in Lymphatic Mapping." Oral presentation at the 33rd Annual Meeting of the Association of Academic Surgery, Philadelphia, PA. November 18-20, Abstract.

4. **Tafra, L,** Lannin L, Egan L, Ramirez M, Watkins D. .(2000) "Accuracy of Sentinel Node Biopsy (SNB) for Large Breast Tumors," Presented at the 82nd Annual Meeting of the American Radium Society, London, England. Abstract.

5. **Tafra L, Verbanac K,** Min CJ, Purser SM, Ng PC, Swanson, MS (2000) "Multicenter Trial of RT-PCR Detection of Sentinel Node Metastases", Dept. of Defense Breast Cancer Research Program ERA OF HOPE, #C23, Proceedings Volume 1, p135.

6. **Verbanac KM**, Min CJ, Lo K, Albrecht J, Purser SM, Swanson MS, Bogey WM and **Tafra L.** (2000) "RT-PCR analysis for mammaglobin and carcinoembryonic antigen detects metastases in histology-negative lymph nodes", to be presented at the 23rd Annual San Antonio Breast Cancer Symposium, December 6-9, Abstract.

7. Chua AC, Lannin DL, Swanson, MS and and **Tafra L.** (2000) "Effect of Technetium 99m Sulphur Colloid Injection Interval on Sentinel Node Biopsy", to be presented at the 23rd Annual San Antonio Breast Cancer Symposium, December 6-9.

8. Lannin D, Cuenca R, Chadwell T, Iheanacho M, **Tafra L.** (2000) "Comparison of three methods for breast lymphatic mapping", to be presented at the 2nd International Sentinel Node Congress, Santa Monica, CA, Dec. 1–4.

Invited Presentations:

Verbanac, KM, Wesley Long Hospital, Greensboro, NC, "Sentinel Node Biopsy: Where have we been and where are we going?" October 5, 1999.

Verbanac, KM, Physicians Cancer Conference, Moses Cone Hospital, Greensboro, NC, "Sentinel Node Biopsy in the Management of Malignancies", October 6, 1999.

Verbanac, KM, East Carolina UnivrsitySchool of Medicine, Culture of Scholarship seminar, "PCR Detection of Micrometastatic Breast Cancer", Octover 2, 2000.

Tafra, L, Grand Rounds, Sentara Virginia Beach General Hospital, Virginia Beach, VA. "Sentinel Node Sampling for Breast Cancer." November 16, 1999.

Tafra, L, Course Director, *"Lymphatic Mapping and Sentinel Node Biopsy: Indications, Techniques and Prospects"* October 22, 1999, January 21, 2000, April 7, 2000, July 14, 2000, November 17, 2000. Breast Center of Anne Arundel Medical Center, Annapolis, Maryland in Reisterstown, Maryland.

B. Funding Applied For Based On Work Supported By This Award

Department of Defense, Breast Cancer Research, Clinical Translational Research Award, Polymerase Chain Reaction and the Detection of Breast Cancer Metastases in Sentinel Lymph Nodes", submitted 8/99, funded 7/00.

CONCLUSIONS

Overall, the impact of sentinel node biopsy on the management of breast caner has been tremendous. The majority (~60%) of breast cancer patients do not have metastases in their lymph nodes. In this population, the ability to replace these the morbidity of an unnecessary axillary node dissection with a simple axillary node dissection is having an impact on patients that is equivalent to the replacement of mastectomy with breast sparing surgery.

There are now many published trials in breast cancer patients that have documented the ability of the sentinel node to predict disease in the remaining lymph node basin. Although the technique of SNB began with melanoma, there are clear differences in performing the technique for breast cancer. The false negative rate for breast cancer patients was significantly higher than for melanoma in the initial series and appears to be due primarily to the fact that the technique has a more difficult learning curve in breast cancer patients.

A crucial component to the success of SLNB in breast cancer patients, is determining those factors that make patients poor candidates for the procedure and those factors that have no bearing on the procedure. We have begun to address these factors, which include age, tumor size and prior surgery. Without this information it remains unclear who is a candidate for axillary sparing SLNB. It is also crucial to resolve many issues that concern the surgical technique itself, such as the site of injection and the timing and preparation of technetium-99m. This clinical trial has begun to discriminate those factors that are important from those that are irrelevant. Only equipped with this data is it possible to move into the next stage of investigating new improvements on the technique to make it globally applicable to all breast cancer patients.

This research is extremely relevant to critical issues in the detection of metastatic breast cancer. The current methods used to detect cancer fail to identify a large number of patients with metastatic disease. Despite the progress made in clinical oncology over the past 20 years, the inability to detect minimal residual disease has hampered efforts to tailor treatment and therefore to improve the quality of life and to, ultimately, decrease mortality. Detection of metastatic disease in a patient newly diagnosed with breast cancer typically relies on a superficial histologic examination of axillary LN. Because this method fails to detect disease in many patients, the physician is forced to either treat histology node negative patients with aggressive therapy (whom might otherwise be treated by minimal intervention) or risk losing patients to sub-clinical disease. Patients being followed after treatment must wait until the development of symptoms or mammographic changes, at which point the chance for significant benefit with intervention is minimal. These studies will assess the prognostic value of new sensitive methods to detect micrometastatic disease in breast cancer patients.

Our of our hypotheses is that PCR analysis for specific, sensitive markers will detect micrometastases in the lymph nodes of breast cancer patients and that this sophisticated level of detection will be a significant predictor of disease-free and overall survival. This proposal is an innovative translational approach to cancer detection that takes advantage of two complementary state-of-the-art techniques: the sensitive analytical technique of RT-PCR and the selective surgical technique of SLNB. More accurate analysis of LN may identify histology node-negative patients at actual risk for recurrence (who are most likely to benefit from aggressive surgery and therapy) and truly node-negative patients (who may not need to be exposed to the risks and morbidity of aggressive surgery or therapy).

Although the clinical relevance of PCR-detected micrometastatic disease has been debated, recent 2-, 3- and 5-year follow-up studies in colon cancer and melanoma indicate that molecular detection of blood or LN is indeed prognostic and of clinical significance. Although the benefit of being able to tailor treatment would be paramount, this work has additional potential significance. It is certainly possible that more accurate detection of sub-clinical nodal involvement (i.e. the detection of occult tumor cells) may actually alter therapy in the future and ultimately may affect survival.

A major strength of this proposal is that it represents a joint collaborative effort between a Ph.D. research scientist and a M.D. surgical oncologist. This work is a concerted effort to unite sophisticated bench research with state-of-the-art surgical management of breast cancer patients in a true bench-to-bedside paradigm. Our collaborative team was among the pioneers who first proposed and investigated the application of SLNB to breast cancer and the application of RT-PCR technology to SLN analysis. We were the first to exhaustively analyze normal LN from non-cancer patients with prospective markers in order to select a panel of truly specific markers for clinical evaluation. We were the first to report the use of mammaglobin, a tissue-specific marker, for the detection of sentinel node metastases. The excellent results we report here for RT-PCR analysis of initial SLN specimens underscores the merit and promise of our approach and we have no doubts that this research will result in substantial improvements in the detection of occult disease.

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1. Tafra L, Lannin D, Swanson M, VanEyk J, Verbanac KM, et al. First multicenter trial of sentinel node biopsy for breast cancer using both technetium sulfur colloid and isosulfan blue. Annals of Surgery 2001; In press.

2. Ng, PC, Chua, AC, Lannin, DP, VanEyk, JJ, Swanson, MS, Tafra, L. Age and surgeon experience: the only significant factors contributing to sentinel node mapping failure in breast cancer, 22nd Annual San Antonio Breast Cancer Symposium, Breast Cancer Research and Treatment. 1999;57:27.

3. Tafra L, Verbanac K, Min CJ, Purser SM, Ng PC, Swanson, MS. Multicenter Trial of RT-PCR Detection of Sentinel Node Metastases, Dept. of Defense Breast Cancer Research Program ERA OF HOPE 2000; #C23, Abstract.

4. Edwards M, Giuliano A, Reintgen D, Tafra L. Consensus statement on guidelines for performance of sentinel lymph node biopsy for breast cancer. 1999; 5 p. American Society of Breast Surgeons: Quarterly.

5. Edwards M, Giuliano A, Reintgen D, Tafra L. Revised Consensus Statement on Guidelines for Performance of Sentinel Lymph Node Biopsy for Breast Cancer. Fall 2000; American Society of Breast Surgeons: Quarterly.

6. Tafra, L., McMasters, K., Whitworth, P., Edwards,M. Credentialing Issues with Sentinel Lymph Node Staging for Breast Cancer. American Journal of Surgery. In press.

7. Tafra, L. State of Affairs of Sentinel Node Biopsy for Breast Cancer. Current Surgery, In press.

8. Chadwell T, Lannin D, Edwards M, et al The gamma probe and isosulfan blue are complementary in accurate lymphatic mapping for early stage melanoma. Abstracts of the 31st meeting of the Association of Academic Surgery 1997; 239 Abstract.

9. Chua A, Tafra L, Edwards M, et al Characteristics of the sentinel node (hot, blue, hot & blue) that determines its significance in lymphatic mapping. Southeastern Surgical Congress 1999; Abstract.

10. Tafra L, Hale JC, Pearsall DW, et al Lymphatic mapping and sentinel node biopsy (SNB) results with prior lumpectomy &biopsy vs. intact tumor. 21st San Antonio Breast Cancer Symposium 1998; *Breast Cancer Research and Treatment* 50(3):257; Abstract.

11. Lannin D, Meade P, Moran J, et al. Is the hottest node the most likely to be positive in lymphatic mapping for soft tissue tumors?. American Society of Clinical Oncology 1998; Abstract.

12. Tafra L, Edwards M, Swanson M, et al. Sentinel node biopsy for breast tumors 4 cm or greater. 1st International Congress on the Sentinel Node in Diagnosis and Treatment of Cancer 1998; Abstract.

13. Kersey, T, VanEyk J, Lannin D, Tafra L. Comparison of Intradermal and Subcutaneous Injections in Lymphatic Mapping, Oral presentation at the 33rd Annual Meeting of the Association of Academic Surgery, Philadelphia, PA 1999; Abstract.

14. Tafra, L, Lannin L, Egan L, Ramirez M, Watkins D. Accuracy of Sentinel Node Biopsy (SNB) for Large Breast Tumors. Presented at the 82nd American Radium Society, London, England. April 2000; Abstract.

15. Chua AC, Lannin DL, Swanson, MS and and Tafra L. Effect of Technetium 99m Sulphur Colloid Injection Interval on Sentinel Node Biopsy", to be presented at the 23rd Annual San Antonio Breast Cancer Symposium, December 6-8 2000; Abstract.

16. Lannin D, Cuenca R, Chadwell T, Iheanacho M, Tafra L. Comparison of three methods for breast lymphatic mapping", to be presented at the 2nd International Sentinel Node Congress, Santa Monica, CA, Dec. 1–4 2000; Abstract.

17. Min CJ, Tafra L, Verbanac KM. Identification of superior markers for PCR detection of breast cancer in sentinel lymph nodes. Oral Presentation, Proc Am Assoc Cancer Res 1998; 39:268 Abstract.

18. Min CJ, Tafra L, Verbanac KM. Identification of superior markers for PCR detection of breast cancer metastases in sentinel lymph nodes. Cancer Research 1998; 58:4581-4584.

19. Verbanac K, Min CJ, Purser S, Tafra L. Mammaglobin, not K19, is a specific marker for RT-PCR detection of breast cancer metastases in sentinel lymph nodes. Society of Surgical Oncology Book of Abstracts 1999; 38:P16 Abstract.

20. Verbanac KM, Fleming TJ, Min CJ, Purser S, Tafra L. RT-PCR increases detection of breast cancer sentinel lymph node micrometastases. San Antonio Breast Cancer Symposium 1999; Abstract.

21. Verbanac KM, Min CJ, Lo K, Albrecht J, Purser SM, Swanson MS, Bogey WM and **Tafra L.** (2000) "RT-PCR analysis for mammaglobin and carcinoembryonic antigen detects metastases in histology-negative lymph nodes", to be presented at the 23rd Annual San Antonio Breast Cancer Symposium, December 7-9.

22. Shivers, S, Stall, A, Trudeau, W, et al. Molecular staging for breast cancer. Proc Amer Assoc Cancer Res 1999;49:495, #3266. Abstract.

23. Leygue E, Snell L, Dotzlaw H, et al. Mammaglobin, a potential marker of breast cancer nodal metastasis. Journal of Pathology 1999; 189:28-33.

24. Shivers SC, Stall A, Fields KK and Reintgen DS. Detection of occult micrometastases in sentinel lymph nodes by RT-PCR. Dept. of Defense Breast Cancer Research Program ERA OF HOPE 2000; #C1. Abstract.

25. Ooka M, Sakita I, Fujiwaa Y, et al. Selection of mRNA markers for detection of lymph node micrometastases in breast cancer patients. Oncology Reports 2000; 7:561-566.

APPENDICES

Reprints/Preprints of key Abstracts and Manuscripts by PIs cited above as Reportable Outcomes.
Curriciulum vita of Dr. Verbanac and Dr. Tafra

CURRICULUM VITAE

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EDUCATION:

B.A., Biology, Kalamazoo College, Kalamazoo, Michigan, 1977

M.S., Molecular, Cellular and Developmental Biology, Iowa State University, Ames, Iowa, 1979.
M.S. Thesis: "Genetic control of preimplantation mouse embryo development by the major histocompatibility complex"
Department of Biochemistry and Biophysics

Ph.D., Biochemistry, University of Iowa, Iowa City, Iowa, 1986.
Ph.D. Thesis: "Biosynthesis, processing and glycosylation of alpha$_1$-antitrypsin"
Department of Biochemistry

EMPLOYMENT HISTORY:

1983-1986	Senior Research Biochemist Bioproducts Laboratory - Central Research The Dow Chemical Company, Midland, Michigan
1986-1989	Project Leader, Cancer Radioimmunotherapy Program Bioproducts Laboratory - Central Research The Dow Chemical Company, Midland, Michigan
1989-1990	Visiting Assistant Professor, Department of Biology East Carolina University, Greenville, North Carolina
1990-1991	Assistant Research Professor, Department of Surgery East Carolina University School of Medicine, Greenville, North Carolina
1991-1994	Instructor, Department of Surgery East Carolina University School of Medicine, Greenville, North Carolina
1994-1996	Assistant Professor, Department of Surgery
1995-present	Adjunct Appointment, Department of Microbiology & Immunology East Carolina University School of Medicine, Greenville, North Carolina
1996-present	Associate Professor, Department of Surgery East Carolina University School of Medicine, Greenville, North Carolina

1998-present Adjunct Appointments, Department of Biology and Department of Physiology
East Carolina University, Greenville, North Carolina

MEMBERSHIP IN PROFESSIONAL SOCIETIES AND PROFESSIONAL SERVICE:

American Association for Advancement of Science
Sigma Xi
Iota Sigma Pi
American Association of Immunologists
American Society of Transplantation
American Association of Cancer Research
Women in Cancer Research
Ad hoc reviewer, *Transplantation*, 1993-present
Ad hoc reviewer, *Leukemia,* 1997-present
Reviewer, Research Subcommittee, American Heart Association, North Carolina Affiliate, 1995-97
Reviewer, American Heart Association Mid-Atlantic Peer Review Consortium Committee, "Cell Transport and Metabolism, Immunology and Microbiology", 1998-present.

GRANTS PREVIOUSLY FUNDED:

Co-investigator, NIH2R01 AI22293, "A Preclinical Model of Allograft Tolerance", National Institutes of Health, $1,580,460, (8/89—3/94).

Principal Investigator, "A Bioengineered Standardized Anti-Human Thymocyte Globulin", North Carolina Biotechnology Center Academic Research Initiation Grant Award, $40,000, (9/91—2/93).

Co-investigator, NIH2R01 AI22293 (year 09-14), "A Preclinical Model of Allograft Tolerance", National Institutes of Health, $1,401,326, (Acting Principal Investigator 6/94-12/94; Co-investigator 12/94-7/95).

Co-investigator, NIH1R01 AI37810-01, "A Preclinical Bridge to Human Transplant Tolerance", National Institutes of Health, (funded 3/95, awarded to the University of Alabama, Birmingham).

Principal Investigator, East Carolina University Starter Grant, "Direct gene transfer of TGF-β to murine cardiac allografts", $3000, (10/95-6/96).

Co-investigator, East Carolina University Starter Grant, "Characteristics of acute natriuretic peptide responses", $3000, (10/95-6/96).

Co-investigator, "Mechanism of dietary effects on vascular hyperplasia in venous aorta coronary bypass grafts," North Carolina Institute of Nutrition, $13,000, (7/96- 6/97).

Principal Investigator, East Carolina University Faculty Research Grant, "Plasmin as a Transforming Growth Factor-beta Activator in Breast Cancer," $15,000, (10/96-6/97).

Co-investigator, East Carolina University Starter Grant, "A strategy for tolerance induction in xenotransplantation," $5,000, (10/96-6/97).

Co-investigator, East Carolina University Faculty Research Grant, "Accuracy and significance of polymerase chain reaction detection of sentinel node metastases in breast cancer patients," $17,000 (10/96-6/97).

Co-Investigator, ECU Leo Jenkins Cancer Center Grant, "Inflammatory breast cancer: a malignancy of neglect or unique biology?" $8000 (awarded 4/97).

Advisor/Sponsor: "Evaluation of Lewis Lung Carcinoma Tumor Growth and Metastasis in Alpha 1.3 galactosyltransferase knock-out mice", $500, Sigma Xi Scientific Research Society (4/97-10/97).

Co-investigator, "Lymphatic Mapping, Sentinel Node Biopsy and Polymerase Chain Reaction Detection of Metastatic Disease in the Breast Cancer Patient ", ECU Faculty Research Grant, $20,360 (6/30/97-6/30/98).

Co-Investigator, "Role of the Vascular Endothelium in Cell Mediated Xenogeneic Responses", ECU Faculty Research Grant, $16,000 (7/30/97-6/30/98).

Principal Investigator, "A Superior Gene for Human Cancer Therapy: α1,3 Galactosyltransferase", VFW Ladies Auxiliary, $2,500 (1997-1998)

Co-investigator, "Improved Detection of Metastases by Lymphatic Mapping", American Cancer Society, East Carolina University Institutional Research Grant, $15,000, 3/98- 9/98.

Co-Investigator, "Chronic immunosuppression in a xenograft model" ECU Starter Research Grant, $5000, 7/30/98-6/30/99.

Principal Investigator, " A superior gene for human cancer therapy: α 1,3 galactosyltransferase" ECU Faculty Research Grant, $25,000, 7/30/98-6/30/99.

ACTIVE GRANTS:

Principal Investigator, "Polymerase Chain Reaction and the Detection of Breast Cancer Metastases in Sentinel Lymph Nodes", U.S. Army Department of Defense Clinical Translational Research Grant, $2,100,000, 7/2000-2004.

Co-Principal Investigator, "Accuracy and Significance of Polymerase Chain Reaction Detection of Sentinel Node Metastases in Breast Cancer Patients", U.S. Army Career Development Grant, $198,440, 9/98-9/01.

Co-Investigator, " Impact of Recipient Cytokine Gene Polymorphisms on Renal Allograft Survival"", $5000, East Carolina University Research Grant, 7/00-6/01.

UNIVERSITY COMMITTEES AND ADMINISTRATIVE ACTIVITIES:

1991-present	• Transplantation Immunology Journal Club, Division of Transplantation, Department of Surgery, East Carolina University
Jul-Dec 1994	• Acting Principal Investigator, NIH2R01 AI22293, "A Preclinical Model of Allograft Tolerance", National Institutes of Health
1994-2000	• East Carolina University Patent and Technology Transfer Committee
1996	• East Carolina University Crime Prevention Task Force - West campus
1996	• Preceptor, Howard Hughes Medical Institute Program for High School Science Teachers
1996	• Member, Institutional Research Grant Group, American Cancer Society, ($187,500 Institutional grant awarded to ECU 7/1/97)
1997-present	• East Carolina University Animal Research Task Force(Ad hoc)
1997-present	• East Carolina University Biological Safety Committee
1999-	• East Carolina University Culture Of Scholarship Committee
	• East Carolina University Research Committee
	• East Carolina University Research Productivity Design Team
	• East Carolina University . Advisory Committee, Interdisciplinary Doctoral Program in Biological Sciences

TEACHING EXPERIENCE:

1977-1979	• Graduate Teaching Assistant, Iowa State University
1979-1983	• Graduate Teaching Assistant, University of Iowa
1986-1989	• Preceptor, Dow Chemical Company / Michigan State University and Central Michigan University Cooperative Student Research Program
1989-1990	• General Biology course, Department of Biology, East Carolina University
1989-present	• Immunobiology Journal Club, Department of Microbiology and Immunology, ECU
1991-1992	• Preceptor, J.H. Rose High School Honors Medical Research Student
1991-present	• Preceptor, ECU Department of Biology and Department of Microbiology and Immunology

	graduate students (Lorita Rebellato awarded Ph.D. 6/95)
	• Preceptor, ECU Department of Surgery residents
1994-present	• Graduate Immunology course, MCBI 6450, Department of Microbiology and Immunology, ECU
1995	• Preceptor, American Heart Association Honors Research student
	• Department of Surgery Grand Rounds, ECU, "Cytokines - A brief review and clinical perspective"
	• Department of Microbiology and Immunology seminar, ECU, "The role of Transforming Growth Factor-beta in the veto mechanism of transplant tolerance"
	• Department of Surgery Research Conference, ECU, "Transforming Growth Factor-beta and the tolerance-promoting effects of donor bone marrow in cardiac allotransplantation"
1995-present	• Full Member, Graduate Faculty, East Carolina University
	• Member, Doctoral student committee, Paula Arnold, Department of Microbiology and Immunology, ECU
1996-1997	• Research advisor, Dr. Brian Hoey, Resident (PGY3), Department of Surgery, ECU
	• Preceptor, American Heart Association Honors Research student
1996-1998	• Master's student co-advisor, Christopher Justus Min, Department of Biology, ECU
	• Member, Doctoral student committee, Paul Hoyle, Department of Microbiology & Immunology, ECU
	• Member Doctoral student committee, Paula Arnold, Department of Microbiology & Immunology, ECU
1996-present	• Medical Microbiology and Immunology, MCBI 6400, Department of Microbiology & Immunology, ECU
	• Graduate Immunology course, MCBI 6450, Department of Microbiology & Immunology, ECU
1996-1999	• Dissertation advisor, Karla Posekany, Department of Microbiology & Immunology, ECU
	• Member, Master's student committee, Samuel Madison, Department of Biology, ECU
1997-present	• Dissertation advisor, Bill Storey, Department of Physiology, ECU
1997	• Preceptor, J. H. Rose High School Medical Honors Student
1998-present	• Master's student advisor, Tia Coleman, Department of Biology, ECU
	• Member, Master's student committee, Brandon Cuthbertson, Department of Biology, ECU
	• Member Doctoral student Ccmmittee, Mindi Walker, Department of Microbiology & Immunology
1998-1999	• Research Advisor, Clint Atkinson, Surgical Resident (PGY3), ECU
1999	• Research advisor, Samantha Steingold (M-1), Summer Research, ECU
1999	• Topics in Applied Immunology, BIO425J, Barton College Immunology Class, Wilson, NC
1999-2000	• Research Advisor, Joseph Franklin, Surgical Resident (PGY3), ECU
	• Research Advisor, Jason Cundiff, Medical student (M3), ECU
2000	• Preceptor, Summer Ventures High School Honors Research Student
	• Member Doctoral student Ccmmittee, Jill Maxwell, Department of Microbiology & Immunology

POSTGRADUATE EDUCATION (last 4 years):

Update on Renal Transplantation, ECU School of Medicine, Greenville, NC, April 15, 1997.

Innovative Approaches to Cancer Treatment, The 21st Annual UNC Lineberger Comprehensive Cancer Center Symposium, Chapel Hill, NC, April 30-May 1, 1997

The Transplantation Society, Fifth Basic Sciences Symposium, Chautauqua, NY, September 6-11, 1997.

89th Annual Meeting, *American Association for Cancer Research,* New Orleans, LA. March,1998

American Society of Transplant Surgeons, Chicago IL May 13-15, 1998.

Update on Renal Transplantation, ECU School of Medicine, Greenville, NC, May 19, 1998

Lymphatic Mapping and Sentinel Node Biopsy: Indications, Techniques, and Prospects in the Management of Malignancies, East Carolina University, Leo Jenkins Cancer Center, Greenville, NC, June 5, 1998.

90th Annual Meeting, *American Association for Cancer Research,* Philadelphia, PA. March 1999

22nd Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 8-11, 1999.

Transplant 2000: Joint meeting of the American Society of Transplant Surgeons, & American Society of Transplantation, Chicago IL, May 2000.

Dept. of Defense Breast Cancer Research Program Meeting, ERA OF HOPE, Atlanta, GA. June 8-11, 2000.

Angiogenesis and Cancer, *American Association for Cancer Research* Special Conference, Traverse City, MI, October 11-15, 2000.

RESEARCH INTERESTS:

PCR detection of micrometastatic disease
Cancer immunology
The vascular endothelial cell and xenotransplantation
Transplant tolerance induction
Antigen processing and presentation
TGF-beta and immunoregulation
Gene therapy

PUBLICATIONS AND PATENTS:

A. Refereed Publications

Verbanac, K.M. and Warner, C.M. (1981) "Role of the major histocompatibility complex in the timing of early mammalian development", in *Cellular and Molecular Aspects of Implantation* (S.R. Glasser and D.W. Bullock, Eds.), New York: Plenum Press, p. 467.

Cozad, K.M., **Verbanac, K.M.**, Goldbard, S.B., and Warner, C.M. (1981) "An automated procedure to measure DNA synthesis in preimplantation mouse embryos", *Gamete Res*, 4:121.

Goldbard, S.B., **Verbanac, K.M.**, and Warner, C.M. (1982) "Role of the H-2 complex in preimplantation mouse embryo development", *Biol Reprod*, 26:591.

Goldbard, S.B., **Verbanac, K.M.**, and Warner, C.M. (1982) "Genetic analysis of H-2 linked gene(s) affecting early mouse embryo development", *J Immunogenet*, 9:77.

Verbanac, K.M. and Heath, E.C. (1983) "Biosynthesis and processing of rat alpha$_1$-antitrypsin", *Arch Biochem Biophys,* 223:149.

Verbanac, K.M. and Heath, E.C. (1986) "Biosynthesis, processing and secretion of M and Z variant human alpha$_1$-antitrypsin", *J Biol Chem*, 261:9979.

Thomas, J.M., **Verbanac, K.M.**, and Thomas, F.T. (1991) "The veto mechanism in transplant tolerance", *Transplantation Reviews*, 5(4):209.

Rebellato, L.M., **Verbanac, K.M.**, Carver, F.M., and Thomas, J.M. (1991) "Immunosuppressive and nonimmunosuppressive rabbit antihuman T lymphocyte antibodies exhibit different recognition patterns to T cell membrane antigens", *Transplant Proc*, 23(1):1117.

Schott, M.E., Frazier, K.A., Pollock, D.K., and **Verbanac, K.M.** (1993) "Preparation, characterization, and *in vivo* biodistribution properties of synthetically crosslinked multivalent anti-tumor antibody fragments", *Bioconjugate Chemistry* 4(3):153-165.

Thomas, J.M., Carver, F.M., Cunningham, P.R., Gross, U., **Verbanac, K.M.**, Rebellato, L., Riley, R. and Thomas, F. T. (1993) "Donor bone marrow infusion suppresses alloantibody response in RATG treated recipients: A correlate of long survival", *Transplant Proc.* 25(1):342-343.

Verbanac, K.M., Gross, U., Rebellato, L.M. and Thomas, J.M. (1993) "Generation of Rabbit Anti-Lymphocyte Monoclonal Antibodies", *Transplant Proc.* 25(1):837-838.

Verbanac, K.M., Gross, U.M., Rebellato, L.M., and Thomas, J.M. (1993) "Production of stable rabbit-mouse heterohybridomas: Characterization of a rabbit monoclonal antibody recognizing a 180 kDa human lymphocyte membrane antigen", *Hybridoma* 12(3):285-295.

Thomas, J.M., **Verbanac, K.M.**, Smith, J.P., Carver, F.M., Kasten-Jolly, J., Gross, U., Rebellato, L.M., Haisch, C.E. and Thomas, F.T. (1994) "Veto cells and the induction of transplant tolerance in primates", in *Rejection and Tolerance - Proceedings of the 25th Conference on Transplantation and Clinical Immunology* (J.L. Touraine, J. Traeger, H. Betrel, J.M. Dubernard, J.P. Revillard and C. Dupuy, Eds.) Kluwer Academic Publishers, The Netherlands, p. 291.

Rebellato, L.R., Gross, U., **Verbanac, K.M.,** and Thomas, J.M. (1994) "A comprehensive definition of the major antibody specificities in polyclonal rabbit anti-human thymocyte globulin", *Transplantation* 57:685-694.

Thomas, J.M., **Verbanac, K.M.,** Carver, F.M., Kasten-Jolly, J., Haisch, C.E., Gross, U. and Smith, J.P. (1994) "Veto cells in transplantation tolerance", *Clinical Transplantation* 8:195-203.

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Thomas, J.M., **Verbanac**, K.M., Smith, J.P., Kasten-Jolly, J., Gross, U., Rebellato, L.M., Haisch, C.E., Carver, F.M. and Thomas, F.T. (1995) "The facilitating effect of one-DR antigen sharing in renal allograft tolerance induced by donor bone marrow in rhesus monkeys", *Transplantation* 59:245-255.

Thomas, J., Kasten-Jolly, J., **Verbanac**, K., Smith, J., Carver, M., Gross, U., Rebellato, L., Thomas, F. and Haisch, C. (1995) "Requirement for DR sharing in stable kidney allograft tolerance induced by donor bone marrow in rhesus monkeys", *Transplant Proc.* 27:166.

Thomas J, Neville, D, Contreras J, Eckhoff D, Meng D, Lobashevsky A, Wang P, Huang Z , **Verbanac K,** Haisch C, and Thomas F. (1997) "Preclinical Studies of Allograft Tolerance in Rhesus Monkeys. A novel anti-CD3-immunotoxin given peritransplant with donor bone marrow induces operational tolerance to kidney allografts", *Transplantation* 64(1):124-135.

Min CJ, Tafra L, **Verbanac KM,** (1998) "Identification of superior markers for PCR detection of breast cancer metastases in sentinel lymph nodes", *Cancer Research* 58(20):4581-4584.

Verbanac,KM, Pittman, HK, Hoey, BA, Rebellato, LM, Araneda, D, Haisch, CE. (1998) "Short Term Antilymphocyte Serum, Rapamycin and Donor Bone Marrow Significantly Suppress Anti-Donor Antibody and Prolong Hamster to Rat Cardiac Xenograft Survival" *Transplantation* 66(8): S49(A-193).

Posekany,KJ, Pittman, HK, Haisch, CE, **Verbanac, KM.** (1998) "The Galactose α 1,3 Galactosyltransferase Knockout Mouse Is Not an Innate Model For Human Hyperacute Rejection; Problems Defined and Solutions Proffered", *Transplantation* 66(8): S69(A-247).

Tafra, L, Lannin,D, Swanson, MS, VanEyk, J, **Verbanac, KM,** et al. (2000) Findings of the first multicenter trial of sentinel node biopsy for breast cancer using both technetium sulfur colloid and isosulfan blue dye*", in press, Annals of Surg.*

B. Book chapters

Thomas, J.M., Thomas, F.T., Emara, M., and **Verbanac, K.M.** (1991) "Mechanisms of cell-mediated rejection", in *Kidney Transplant Rejection* (J. Burdick, Ed.), Marcel Dekker, Inc., p. 21.

Thomas, J.M. and **Verbanac K.M.** (1996) "Tolerance induction: Basic concepts and therapeutic approaches" in *Principles of Drug Design in Transplantation and Autoimmunity* (R. Lieberman, Ed.) Raven Press, New York.

Haisch CE and **Verbanac, KM**. (1998) "Immunity and the Immunocompromised Patient", in *Physiological Basis of Modern Surgical Care (*Thomas Miller, Ed.) Quality Medical Publishing. St. Louis, MO.

C. Patents and Disclosures

Anderson, W.H.K.A. and **Verbanac, K.M.** (1989) "New monoclonal antibodies recognizing TAG 72, a human carcinoma antigen", U.S. Patent Disclosure 37,577 (not filed).

Kaplan, D.A., Goeckeler, W.F., and **Verbanac, K.M.** (1990) "Use of colony stimulating factor in combination with bone agent compositions for the treatment of cancer", U.S. Patent 38,475.

D. Abstracts

Verbanac, K.M. and Heath, E.C. (1982) "Biosynthesis and processing of rat alpha$_1$-antitrypsin", *Fed Proc,* 41:517.

Verbanac, K.M. and Heath, E.C. (1983) "In vitro biosynthesis and processing of the ZZ variant form of human alpha$_1$-antitrypsin", *Fed Proc,* 42:1949.

Rebellato, L.M., **Verbanac, K.M.**, Carver, F.M., and Thomas, J.M. (1990) "Immunosuppressive and nonimmunosuppressive rabbit antihuman T lymphocyte antibodies exhibit different recognition patterns to T cell membrane antigens", *The Transplantation Society, 13th International Congress Book of Abstracts*, p. 488.

Anderson, W.H.K., Goeckeler, W.F., Harrington, C.K., Spittka, G.A., Stoneburner, L.K., and **Verbanac, K.M.** (1991) "Modification of biodistribution of radiolabeled CC49 in tumor bearing mice with anti-idiotypic antibodies", *J Nucl Med*, 32(5):1058.

Verbanac, K.M., Gross, U., Rebellato, L.M. and Thomas, J.M. (1992) "Generation of rabbit anti-human lymphocyte monoclonal antibodies", *The Transplantation Society, 14th International Congress Book of Abstracts*, p. 477.

Thomas, J.M., Carver, F.M., Cunningham, P.R., **Verbanac, K.M.**, and Thomas, F. T. (1992) "Donor bone marrow infusion suppresses alloantibody response in RATG treated recipients", *The Transplantation Society, 14th International Congress Book of Abstracts*, p. 257.

Verbanac, K.M., Carver, F.M., Haisch, C.E. and Thomas, J.M. (1993) "Transforming Growth Factor-Beta (TGF-β) May Function in the Veto Mechanism in Transplant Tolerance", *American Society of Transplant Surgeons, 19th Annual Meeting Book of Abstracts*, p. 141, #F1.

Thomas, JM, Kasten-Jolly, J, Smith, J, **Verbanac, K**, Carver, M, Gross, U, Rebellato, L, Thomas, F. (1994) "MHC Class II Sharing Requirement for Stable Renal Allograft Tolerance Induced by Donor Bone Marrow in Rhesus Monkeys," *American Society of Transplant Surgeons 20th Annual Meeting Book of*

Abstracts, p. 181, #H1.

Kasten-Jolly, J., Smith, J., **Verbanac, K.**, Haisch, C., Carver, M. and Thomas, J. (1994) "Factors in successful tolerance induction with post-transplant TLI and donor bone marrow," *15th World Congress of The Transplantation Society Book of Abstracts*, p. 113, #182.

Thomas, JM, Kasten-Jolly, J, **Verbanac, K**, Carver, M, Gross, U, Rebellato, L, Thomas, F, Haisch, C. (1994) "Requirement for DR Sharing in Stable Kidney Allograft Tolerance Induced by Donor Bone Marrow in Rhesus Monkeys," *15th World Congress of The Transplantation Society Book of Abstracts*, p. 113, #181.

Thomas, J., Rebellato, L., Haisch, C., **Verbanac, K.**, Thomas, F.T., Cavender, D.E., Knowles, R.W. (1995) "Prolonged administration of humanized anti-CD4 monoclonal antibody OKTcdr4A to induce long term allograft unresponsiveness in rhesus monkeys", *American Society of Transplant Surgeons, 21st Annual Meeting Book of Abstracts*, p. 53.

Verbanac, KM, Pittman, HK, Haisch, CE. (1996) "Stimulation of xenogeneic in vitro responses: Direct presentation by murine microvascular endothelial cells (VEC) to human T helper lymphocytes", Annual Meeting of the American Association of Immunologists, *Program Addendum and Late-Breaking Book of Abstracts*, p. 51.

Thomas, J, Haisch, C, Eckhoff, D, Lobashevsky, A, Contreras, J, **Verbanac, K,** Carver, M, Thomas, F. (1997) "Stable Long-Term (1-5 Year) Survival Of Kidney Transplants In A Preclinical Model Without Chronic Immunosuppressive Drugs or Pretransplant Treatment", 23rd Annual Scientific Meeting of the American Society of Surgeons, *Program and Book of Abstracts,* p. A-329

Verbanac KM, Pittman HK, Haisch CE. (1997) "Xenogeneic Mixed Lymphatic Endothelial Cell (MLEC) Cellular Responses May Be Mediated By A Non-Class II Xenoantigen", The Transplantation Society, Fifth Basic Sciences Symposium, *Fifth Basic Sciences Symposium of the Transplantation Society, Program and Poster Abstracts.*

Thomas JM, Haisch C, Contreras J, Lobashevasky A, Eckhoff D, **Verbanac K,** Carver M, Thomas, F. (1997) "Stable Long Term (1-5 Year) Survival of Kidney Transplants in a Preclinical Model Without Chronic Immunosuppressive Drugs or Pretransplant Treatment", The Transplantation Society, Fifth Basic Sciences Symposium, *Fifth Basic Sciences Symposium of the Transplantation Society, Program and Poster Abstracts*

Min CJ, Tafra L, **Verbanac KM**. (1998) "Identification of superior markers for PCR detection of breast cancer metastases in sentinel lymph nodes", Annual Meeting, *American Association for Cancer Research.,* Abstract #1883.

Verbanac,KM, Pittman, HK, Hoey, BA, Rebellato, LM, Araneda, D, Haisch, CE. (1998) "Short Term Antilymphocyte Serum, Rapamycin and Donor Bone Marrow Significantly Suppress Anti-Donor Antibody and Prolong Hamster to Rat Cardiac Xenograft Survival" *Transplantation* 66(8):S49 (A-193).

Posekany,KJ, Pittman, HK, Haisch, CE, **Verbanac, KM.** (1998) "The Galactose α 1,3 Galactosyltransferase Knockout Mouse Is Not an Innate Model For Human Hyperacute Rejection Problems Defined and Proffered", *Transplantation* 66(8):S49 (A-247).

Posekany, K, Pittman, K, Swanson, M, Haisch, C, **Verbanac, K.** (1999), "α1,3 Galactosyltransferase (GalT) Expression by Tumor Cells Presents Targets for Antibody-mediated Tumor Destruction in GalT KO Mice: Implications for Human Cancer Gene Therapy" *Proceedings of the American Association for Cancer Research 40:490, #3233.*

Verbanac, KM, Min, CJ, Purser, S, Tafra, L . (1999) " Mammaglobin, Not K19, Is a Specific Marker for RT-PCR Detection of Breast Cancer Metastases in Sentinel Lymph Nodes (SLN)", *Society of Surgical Oncology Program Book of Abstracts,* 38: P16.

Verbanac, KM, Fleming, T, Min, CJ, Purser, S, Tafra, L. (1999) " RT-PCR increases detection of breast cancer sentinel lymph node micrometastases", *Breast Cancer Research & Treatment* 57:41, #125.

Coleman, T, Pittman, HK, Purser, S, Haisch, CE, Verbanac, KM, (2000) "Human anti-porcine aortic endothelial cell in vitro responses: de novo induction of SLA Class II and VCAM-1", *Transplantation* 69(8):S256, #559.

Storey BT, Pittman HK, Min CJ, Haisch CE, Verbanac KM. (2000) "Murine Vascular Endothelial Cells Specifically Home to Syngeneic Lung Adenocarcinomas and Incorporate into Tumor Vasculature", ***American Association of Cancer Research Special Conference : Angiogenesis and Cancer Book of Abstracts*, #B-45.**

PRESENTATIONS:

The National Institute of Child Health and Human Development and the Center for Population Research, Sponsored Conference on Cellular and Molecular Aspects of Implantation, Houston, Texas, "Role of the major histocompatibility complex in the timing of early mammalian development", September 17, 1979.

Federation of American Societies for Experimental Biology and Guest Societies, 66th Annual Meeting, New Orleans, Louisiana, "Biosynthesis and processing of rat alpha$_1$-antitrypsin", April 15-23, 1982.

American Society of Biological Chemists, 74th Annual Meeting, San Francisco, California, "In vitro biosynthesis and processing of the ZZ variant form of human alpha$_1$-antitrypsin", June 5-9, 1983.

The Dow Chemical Company Central Research Spring Scientific Meeting, Midland, Michigan, "Radioimmunotherapy - A New Approach to Soft Tissue Metastatic Disease: In vivo biodistribution and metabolism of immunoconjugates", March 18, 1989.

The Transplantation Society, 13th International Congress, San Francisco, California, "Immunosuppressive and nonimmunosuppressive rabbit antihuman T lymphocyte antibodies exhibit different recognition patterns to T cell membrane antigens", August 19-24, 1990.

The Society of Nuclear Medicine, 38th Annual Meeting, Cincinnati, Ohio, "Modification of biodistribution of radiolabeled CC49 in tumor bearing mice with anti-idiotypic antibodies", June 11-14, 1991.

The Transplantation Society, 14th International Congress, Paris, France, "Generation of rabbit anti-human lymphocyte monoclonal antibodies", August 16-21, 1992.

The Transplantation Society, 14th International Congress, Paris, France, "Donor bone marrow infusion suppresses alloantibody response in RATG treated recipients", August 16-21, 1992.

American Society of Transplant Surgeons, 19th Annual Meeting, Houston, Texas, "Transforming Growth Factor-Beta (TGF-β) May Function in the Veto Mechanism in Transplant Tolerance", May 19-21, 1993.

American Society of Transplant Surgeons, 20th Annual Meeting, Chicago, IL, "MHC Class II sharing requirement for stable renal allograft tolerance induced by donor bone marrow (DBM) in rhesus monkeys", May 18-20, 1994.

The Transplantation Society, 15th International Congress, Kyoto, Japan, "Requirement for DR Sharing in Stable Kidney Allograft Tolerance Induced by Donor Bone Marrow in Rhesus Monkeys." August 28-September 2, 1994.

The Transplantation Society, 15th International Congress, Kyoto, Japan, "Factors in Successful Tolerance Induction with Post-transplant TLI and Donor Bone Marrow ", August 28-September 2, 1994.

21st Annual Meeting of the American Society of Transplant Surgeons, Chicago, IL "Prolonged administration of humanized anti-CD4 monoclonal antibody OKTcdr4A to induce long term allograft unresponsiveness in rhesus monkeys", May 17-19, 1995.

American Association of Immunologists, Annual Meeting, New Orleans, LA, "Stimulation of xenogeneic in vitro immune responses: Direct presentation by murine microvascular endothelial cells to human T helper lymphocytes", June 1-6, 1996.

East Carolina University School of Medicine, Department of Surgery Research Day, Greenville, NC, "Stimulation of Xenogeneic In Vitro Immune Responses: Direct Presentation By Murine Microvascular Endothelial Cell To Human T Helper Lymphocytes", September 7, 1996.

American Cancer Society Institutional Research Grant Site Visit, East Carolina University, "Plasmin as a Transforming Growth Factor-beta Activation in Breast Cancer" September 17, 1996.

East Carolina University Doctoral Student Research Day, "Normal Human Serum Induces Cell Death In Lewis Lung Carcinoma Cells That Express The Alpha 1,3 Gal Epitope", January 1997.

The 23rd Annual Scientific Meeting of the American Society of Surgeons, Chicago, IL, "Stable Long-Term (1-5 Year) Survival Of Kidney Transplants In A Preclinical Model Without Chronic Immunosuppressive Drugs or Pretransplant Treatment", May 14-16, 1997.

Lymphatic Mapping and Sentinel Node Biopsy: Indications, Techniques, and Prospects in the Management of Malignancies, Greenville, NC, "Potentials and Pitfalls of Polymerase Chain Reaction Technique", May 28, November 14, 1997.

The Transplantation Society, Fifth Basic Sciences Symposium, Chautauqua, NY, "Xenogeneic Mixed Lymphatic Endothelial Cell (MLEC) Cellular Responses May Be Mediated By A Non-Class II Xenoantigen", September 6-11, 1997.

The Transplantation Society, Fifth Basic Sciences Symposium, Chautauqua, NY, "Stable Long Term (1-5 Year) Survival of Kidney Transplants in a Preclinical Model Without Chronic Immunosuppressive Drugs or Pretransplant Treatment", September 6-11, 1997

The Society of Microsurgical Specialists, San Antonio, Texas, "A Refined Technique for Murine Heterotopic Cardiac Transplantation", October 16-18, 1997.

American Association for Cancer Research Annual Meeting, New Orleans, LA, "Identification of superior markers for PCR detection of breast cancer metastases in sentinel lymph nodes", March 30, 1998.

East Carolina University Doctoral Student Research Day, "Induction of anti-gal antibodies in galT knock-out mice and their potential for anti-tumor Activity", April 13, 1998.

Seminar Speaker for the Department of Physiology, ECU, "Galactose α 1,3 galactosyltransferase for cancer gene therapy: Harmonic convergence of transplantation and cancer immunology", April 28, 1998.

American Society of Transplant Surgeons, Chicago IL "Short Term Antilymphocyte Serum, Rapamycin and Donor Bone Marrow Significantly Suppress Anti-Donor Antibody and Prolong Hamster to Rat Cardiac Xenograft Survival, May 13, 15, 1998.

American Society of Transplant Surgeons, Chicago IL "The Galactose α 1,3 Galactosyltransferase Knockout

Mouse Is Not an Innate Model For Human Hyperacute Rejection: Problems Defined and Proffered", May 13, 15, 1998.

Lymphatic Mapping and Sentinel Node Biopsy: Indications, Techniques, and Prospects in the Management of Malignancies, Greenville, NC, "Potentials and Pitfalls of Polymerase Chain Reaction Technique", February 27, June 5, August 14, 1998, December 4, 1998.

East Carolina University Department of Physics Colloquium, "Reverse Transcriptase-Polymerase Chain Reaction for the Detection of Breast Cancer Micrometastases in Sentinel Lymph Nodes: The Identification of Specific Markers", October 30, 1998.

The 52nd Annual Meeting of the Society of Surgical Oncology, Inc, Orlando FL, "Mammaglobin, Not K19, Is A Specific Marker For RT-PCR Detection Of Breast Cancer Metastases In Sentinel Lymph Nodes (SLN)", March 4-7, 1999.

90th Annual Meeting of The American Association of Cancer Research, Philadelphia, PA, " α1,3 Galactosyltransferase (GalT) Expression by Tumor Cells Presents Targets for Antibody-mediated Tumor Destruction in GalT KO Mice: Implications for Human Cancer Gene Therapy", April 10-14, 1999.

East Carolina University Microbiology and Immunology Conference, "Carbohydrates Aren't Just for B-cells Anymore: MHC-restricted T-Cell Recognition of Glycopeptides", April 28, 1999

Wesley Long Hospital, Greensboro, NC, "Sentinel Node Biopsy: Where have we been and where are we going?" October 5, 1999.

Moses Cone Hospital, Greensboro, NC, "Sentinel Node Biopsy in the Management of Malignancies", Physicians Cancer Conference, October 6, 1999.

San Antonio Breast Cancer Symposium, San Antonio, TX, "RT-PCR increases detection of breast cancer sentinel lymph node micrometastases", December 7-12, 1999.

East Carolina University Doctoral Student Research Day, "The role of cellular adhesion molecules in vascular endothelial cell homing to tumor neovasculature", April 3, 2000.

Transplant 2000, Chicago, IL "Human anti-porcine aortic endothelial cell in vitro responses: de novo induction of SLA Class II and VCAM-1", *Transplant 2000*. May 13-17, 2000.

Dept. of Defense Breast Cancer Research Program ERA OF HOPE, Atlanta, GA, "Multicenter Trial of RT-PCR Detection of Sentinel Node Metastases", June 8-11, 2000.

Angiogenesis and Cancer, *American Association for Cancer Research* Special Conference, "Murine Vascular Endothelial Cells Specifically Home to Syngeneic Lung Adenocarcinomas and Incorporate into Tumor Vasculature, "Traverse City, MI, October 11-15, 2000.

East Carolina University School of Medicine, Culture of Scholarship seminar, "PCR Detection of Micrometastatic Breast Cancer", October 2, 2000.

SPECIAL HONORS:

Elected to Iota Sigma Pi, 1979
Elected to Sigma Xi, 1993
Elected as Secretary, Greenville chapter, Sigma Xi, 1996-98; Appointed to Executive Committee 1998
Elected to American Association of Immunologists, 1995

Appointed, ECU Clinical Transplant Team, 1996
Appointed, American Heart Association, NC Affiliate, Research Review Subcommittee, 1995-97
Appointed, American Heart Association Mid-Atlantic Peer Review Consortium Committee, "Cell Transport and Metabolism, Immunology and Microbiology", 1997-present
Elected to American Society of Transplantation 1998
Elected to American Association of Cancer Research 1998
Appointed to East Carolina University Research Productivity Design Team 1998

COMMUNITY SERVICE:

Preceptor, J.H. Rose High School Honors Student in Medical Science, 1991-1992, 1997-1998.
American Heart Association
Volunteer, 1992-94
Preceptor, American Heart Association Honors Research student, 1995, 1997
Our Redeemer Lutheran Church
Lector, 1991-present
Member of Social Ministry Committee, 1992-1995
Sunday School teacher, 1995-1997
Church Council member, 1999-
Habitat for Humanity, 1992-present
Greenville Museum of Art, member, 1995-present
Preceptor, EXODUS program, Pitt County Schools, 1996
Preceptor, J.H. Rose High School student, Science Fair project, 1996
Preceptor, Summer Ventures program, high school science student, 1996, 2000
Preceptor, Howard Hughes Medical Institute program, J.H. Rose High School science teacher, 1996
Speaker, J.H. Rose High School Science Journal Club, 1996
Judge, J.H. Rose High School Science Fair, 1997-present
Speaker, "Prescription for Science Literacy" Workshops for elementary, middle and high school science teachers in the North Carolina public schools (Jan 16, February 16, February 27, and March 16, 1998).
Speaker, "Galactose α 1,3 galactosyltransferase for gene therapy", Chamber of Commerce Leadership Network, December 15, 1998

Updated 10/2000

CURRICULUM VITAE

Lorraine Tafra, MD

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Education

	Graduated	Degree
Stuyvesant High School New York, New York	1976	
Skidmore College Saratoga, New York	1981	BA
Case Western Reserve Medical School Cleveland, Ohio	1986	MD

Surgical Residency Training

	Dates
Intern, General Surgery Brown University Providence, Rhode Island	July 86 - June 87
Assistant Surgical Resident, General Surgery Brown University Providence, Rhode Island	July 87 - December 88
Assistant Surgical Resident, General Surgery Thomas Jefferson University Philadelphia, Pennsylvania	July 90 - June 91
Chief Surgical Resident, General Surgery Thomas Jefferson University Philadelphia, Pennsylvania	July 91 - June 92

Clinical Fellowship

John Wayne Cancer Institute - Surgical Oncology Santa Monica, California	July 92 - June 94

Appointments

Director, Breast Center of Anne Arundel Medical Center Annapolis, Maryland	July 99 - Present
Assistant Professor of Surgery East Carolina School of Medicine Greenville, North Carolina	July 95 - May 99
Assistant Clinical Professor of Surgery University of Pennsylvania Philadelphia, Pennsylvania	March 95 - June 96
Surgical Staff Jeanes Hospital Philadelphia, Pennsylvania	September 94 - August 95

Surgical Staff Holy Redeemer Hospital Meadowbrook, Pennsylvania	January 95 - August 95
Clinical Instructor in Surgery University of Southern California Los Angeles, California	July 92 - June 94
Surgical Staff Century City Hospital Los Angeles, California	February 94 - June 94
Surgical Staff St. John's Hospital Santa Monica, California	July 92 - June 94

Research

	Dates
Research Fellow John Wayne Cancer Institute Santa Monica, California	July 93 - June 94
Harrison Department of Surgical Research Fellow University of Pennsylvania Philadelphia, Pennsylvania	January 89 - June 90
NIH Immunology Training Grant Fellow University of Pennsylvania Philadelphia, Pennsylvania	June 89 - June 90
Research Assistant in Department of Pathology Hospital for Special Surgery New York, New York	June 80 - April 82
Research Technician in Department of Hematology Cornell University New York, New York	June 80 - June 82
Research Volunteer in Department of Orthopedics Hospital for Special Surgery New York, New York	June 79 - August 79

Licensure

Pennsylvania
California
North Carolina

Specialty Board Certification

American Board of Surgery — February 1994
Certificate # 38964

Hobbies & Interests

Alpine skiing, Tennis

Committees

Research and Training Committee, American Society of Breast Surgeons
Director, Breast Center Executive Committee
ECU Interdepartmental Breast Cancer Research Retreat, Co-Chair
Senator for the ECU Faculty Senate
Cancer Committee
Leo W. Jenkins Cancer Center Annual Fund Raiser Gala, Co-Chair 1997
Medical Director, *Made in Shade* Melanoma Support Group
Surgical Director, Melanoma Clinic,

Professional Society Memberships

American College of Surgeons
The American Society of Breast Surgeons
American Society of Clinical Oncology
Society of Surgical Oncology
Association of Women Surgeons
American Association for Cancer Research
Association for Academic Surgery
North Carolina Medical Society
American Federation for Medical Research

Honors / Awards

Phi Beta Kappa
Berlex Oncology Foundation / Clinical Trial Design — August 1994
Duke Research Symposium Award for Presentation on the first clinical series in North Carolina of sentinel node biopsy for melanoma presented by ECU surgical resident — February 1997
Research Award to Dr. Arlene Chua, research resident for presentation at the North Carolina Chapter of American College — August 1999

Editorial Board

Current Surgery 1996 - Present

Publications

Tafra, L., McMasters, K., Whitworth, P., Edwards,M. SentinelLymph Node Staging for Breast Cancer: The Credentialing Issue. *In press* American Journal of Surgery. December 2000.

Tafra, L. State of Affairs of Sentinel Node Biopsy for Breast Cancer. *In press* Current Surgery. November 2000.

Edwards M, Giuliano A, Reintgen D, **Tafra L.** Revised Consensus Statement on Guidelines for Performance of Sentinel Lymph Node Biopsy for Breast Cancer. *American Society of Breast Surgeons: Quarterly*; Fall 2000.

Tafra, L. Will the True Sentinel Node Please Stand. Letter to the Editor Annals of Surgery, 6(5),1999; 514-516.

Tafra, L., Lannin, D.R., Verbanac, K.M., et. al. First Multi-center Trial of Sentinel Node Biopsy Using Isosulfan Flue and Tc99-m for Breast Cancer: Factors Associated with Success. Submitted Annals of Surgery, September, 1999.

Williamson, J.D., Silverman, J.F., **Tafra, L.** Fine Needle Aspiration of Metastatic Squamous Cell Carcinoma Arising in a Pilonidal Sinus with a Review of the Literature. *In press* Diagnostic Cytopathology, June, 1998.

Edwards M, Giuliano A, Reintgen D, Tafra L. Consensus Statement on Guidelines for Performance of Sentinel Lymph Node Biopsy for Breast Cancer. *American Society of Breast Surgeons: Quarterly* 1998; Fall: 3.

Minn, C.J., **Tafra, L.,** Verbanac, K.M. Identification of Superior Markers for PCR Detection of Breast Cancer Metastases in Sentinel Lymph Nodes. Cancer Research., Vol. 58, October, 1998, pp. 4581-4584.

McMasters, K.M., Giuliano, A.E., Ross, M.I., Reintgen, D.S., Hunt, K.K., Byrd, D.R., **Tafra, L.,** Klimberg, S.V., Whitworth, P.W., Edwards, M.J. Sentinel Lymph Node Staging for Breast Cancer: Not Yet Standard of Care. New England Journal of Medicine, Vol.339, No.14, October 1, 1998, pp. 990-995.

Tafra, L., Chua, A.N., Ng, P.C., Aycock, D., Swanson, M., Lannin, D.: Filtered versus Unfiltered Technetium Sulfur Colloid in Lymphatic Mapping in a Pig Model. Annals of Surgical Oncology, Vol. 6 (1), March, 1999, pp. 83-87.

Tafra L. Selective Lymph Node Dissection for the Management of Malignancies. Current Surgery, Vol. 53:2, Feb. 1996.

Tafra L., Dale, P, Giuliano AE. Nonpalpable vs. Palpable Invasive Breast Tumors Treated with Breast-Conserving Surgical Management. American Surgeon, 62(5):395 May, 1996.

Tafra L, Dale P, Wanek L, Ramming K, Morton, D: Resection and Adjuvant Immunotherapy for Melanoma Metastatic to the Lung and Thorax. Journal of Thoracic and Cardiovascular Surgery, Vol. 110:119 - 29, 1995.

Spivac B, Khanna MM, **Tafra L**, Jiullard G, Giuliano AE: Margin Status and Local Recurrence After Breast-Conserving Surgery. Archives of Surgery, Vol. 129: 952-7, 1994.

Tafra L , Chang C, Foshag L , Morton DL: Long-Term Survivors of Melanoma Metastatic to Distant Sites. Clinical Oncology, *in press*.

Tafra L, Guenther M , Giuliano A E: Planned Segmentectomy: A Necessity in the Management of Breast Carcinoma. Archives of Surgery, Vol. 128: 1014-8, September 1993.

Dafoe DC, Wang X, **Tafra L**, Berezniak R, Lloyd RV.: Studies of Composite Grafts of Fetal Pancreas(FP) and Fetal Liver(FL) in the Streptozotocin-Induced Diabetic Rat. Advances in Experimental Medicine & Biology, Vol.321: 171-7, 1992.

Wang X, Berezniak R, **Tafra L**, Posselt A, Barker CF, Dafoe DC : Intraportal FK 506 Improves Intrahepatic Islet Allograft Survival. Transplantation Proceedings, Vol. 23(6): 3211-2, 1991.

Wang XG, **Tafra L**, Berezniak R, Lloyd RV, Muraika L, Dafoe DC : Effects of Cotransplanted fetal liver on fetal pancreas isografts. Transplantation Vol. 53(2): 272-6, Feb. 1992.

Tafra L, Dafoe DC, Berezniak R: Fetal Liver and Pancreas Transplanted as a Composite Improves Islet Graft Function. Transplantation Proceedings, Vol. 23(1): 752-3, 1991.

Tafra L, Dafoe DC, Berezniak R: Beneficial Effects of Fetal Liver Tissue on Fetal Pancreas Transplants. Surgery Vol. 108: 734-40, Oct. 1990.

Dafoe CD, Smythe WR, Berezniak R, **Tafra L**, Shaw LM, Tomaszewski JE, Barker CF: An Innovative Site for Fetal Pancreas Transplantation in Rats - The Subserosa of a "U-Loop" of Small Intestine. Transplantation Vol. 48(5): 863-5, 1989.

Bullough P, Yawit, P, **Tafra L** : Topographical Variations on the Morphology and Biochemistry of Adult Canine Tibial Plateau Articular Cartilage. Journal of Orthopedic Research. 3:l-16, l985.

Lane J, Warren R, Gartsman G, Sculco T, **Tafra L**: Pathological Fractures of the Humerus; An Epidemiological Study and an Evaluation of the Sampson Rod. Orthopedic Transactions. Vol. 6:l, l982.

Chapters

Dafoe DC, Wang X, **Tafra L**, Berezniak R, Lloyd R: ***Studies of Composite Grafts of Fetal Pancreas(FP) and Fetal Liver(FL) in the Streptozotocin-Induced Diabetic Rat.***. In Vinik, AI(Ed.), Pancreatic Islet Cell Regeneration and Growth, Plenum Press, New York, New York,1992.

Abstracts

"Effect of Technetium Sulphur Colloid Injection Interval on Sentinel Node Biopsy". To be presented at 23rd Annual San Antonio Breast Cancer Symposium, San Antonio. December 6-8,2000.

"Comparison of Three Methods for Breast Lymphatic Mapping". Submitted to 2nd International Sentinel Node Congress, San Monica, CA. August 25, 2000.

"Multi-Center Trial of RT-PCR Detection of Sentinel Node Metastases". Presented at Department of Defense Breast Cancer Research Program: Era of Hope, for Atlanta, Georgia. June 2000. Submitted January 2000.

"Accuracy of Sentinel Node Biopsy (SNB) for Large Breast Tumors." Submitted to 82nd American Radium Society, for London, England. April 2000. Submitted September 1999.

"Age and Surgeon Experience: The Only Significant Factors Contributing To Sentinel Node Mapping Failure In Breast Cancer." Presented at 22nd Annual San Antonio Breast Cancer Symposium. San Antonio.December 8, 1999. Breast Cancer Research and Treatment, Vol. 57, NO, 1,1999, pp 27.

"RT-PCR Increases Detection of Breast Cancer Sentinel Lymph Node (SLN)Micrometastases Presented at 22nd Annual San Antonio Breast Cancer Symposium, for San Antonio. December 1999.

"Comparison of Intradermal (ID) and Subcutaneous (SC) Injections in Lymphatic Mapping." Submitted to 33rd Annual Meeting of the Association of Academic Surgery, for Philadelphia, PA. November 1999. Accepted for oral presentation.

"Surgeon Experience: The Only Significant Factor Contributing to Sentinel Node Mapping Failure in Breast Cancer in a Multi-Center Trial" Presented at the North Carolina Chapter of the American College of Surgeons, Asheville, N.C. July 1999.

"Differential lymphatic mapping: a method using multiple lymphotropic dyes." Presented by student MaryJoy Iheanacho, National Student Research Forum, Galveston Texas. April 1999.

"Timing of Technetium Sulfur Colloid (Tc99) Injection does not Alter the Results of Sentinel Node Biopsy (SNB) for Breast Cancer." Accepted: 1st International Congress on the Sentinel Node in Diagnosis and Treatment of Cancer, December 1998.

"Sentinel Node Biopsy (SNB) for Breast Tumors 4 cm or Greater" Accepted: 1st International Congress on the Sentinel Node in Diagnosis and Treatment of Cancer, December 1998.

"Mammoglobin, not K-19, is a specific marker for RT-PCR detection of breast cancer metastases in sentinel lymph nodes." Presented at the Society of Surgical Oncology, March 1999.

"Differential lymphatic mapping: a method using multiple lymphotropic dyes." Accepted American Association of Cancer Research, September 1998.

"Prior experience in the only significant factor associated with sentinel node mapping failure in breast cancer." Presented at the Society of Surgical Oncology, September 1998.

"Lymphatic Mapping and sentinel node biopsy (SNB) results with prior lympectomy and biopsy vs. intact tumor." presented at the 21st Annual San Antonio Breast Cancer Symposium, San Antonia. December 13, 1998.Breast Cancer Research and Treatment, pp 257.

"Characteristics of the Sentinel Node (Hot, Blue, Hot & Blue) that Determines its Significance in Lymphatic Mapping." Accepted Southeastern Surgical Congress, July 1998.

"Is the hottest node the node most likely to be positive in lymphatic mapping for soft tissue tumors?" Presented at the American Society of Clinical Oncology Los Angeles. May 1998.

"Identification of superior markers for PCR detection of breast cancer metastases in sentinel lymph nodes." Presented at the American Association of Cancer Research. New Orleans. March 1998.

"Filtered vs. Unfiltered Technetium Sulfur Colloid in Lymphatic Mapping in a Pig Model." Presented at the Society of Surgical Oncology. San Diego. March 1998.

"The Gamma Probe & Isosulfan are Complementary in Accurate Lymphatic Mapping for melanoma." Association of Academic Surgery. Dallas. November 1997.

"Selective (Sentinel) Lymph Node Biopsy for Melanoma." Fifth Annual North Carolina Duke Cancer Research Symposium. February 1997.

"Breast Cancers Found by the Physician During Routine Examination." Society of Surgical Oncology. Atlanta, Georgia. March 1996.

"Breast-Conserving Surgery in Young Women with Breast Cancer." Society of Surgical Oncology. Houston, Texas. March 1994.

"Management and Prognosis of Pulmonary Melanoma." American Society of Thoracic Surgery. New York, New York. April 1994.

"Breast Conserving Surgery for Mammographically Detected versus Palpable Invasive Carcinomas." American Radium Society. Aruba. April 1993.

"Long-Term Survivors of Melanoma Metastatic to Distant Sites." Society of Surgical Oncology. Los Angeles, California. March 1993.

"Effect of Surgical Margins and Radiation Dose on Local Recurrence after Breast - Conserving Surgery for Invasive Carcinoma", Society of Surgical Oncology. Los Angeles, California. March 1993.

"Planned Segmentectomy: A Necessity in the Management of Breast Carcinoma." Pacific Coast Surgical Association. Scottsdale, Arizona. February 1993.

"Small Bowel Subserosa as a Superior Site for Fetal Pancreas Transplantation Compared to the Renal Subcapsule Site." Association of Academic Surgery. Houston, Texas. November 1990.

"Fetal Liver and Pancreas Transplanted as a Composite Improves Islet Graft Function." XIII International Congress of the Transplantation Society. San Francisco, California. August 1990.

"Beneficial Effects of Fetal Liver Tissue on Fetal Pancreas Transplants." Central Surgical Association. Chicago, Illinois. March, 1990.

"Effects of Local Delivery of Cyclosporine on Transplanted Islets." The Society of University Surgeons Residents' Program. Los Angeles, California. February, 1990.

"Successful Fetal Pancreas Transplantation to an Innovative Small Bowel Site in Rats - The "U-Loop."" Second International Congress on Pancreatic and Islet Transplantation. Minneapolis, Minnesota. September, 1989.

Presentations (National and Regional Meetings only)

"Characteristics of the Sentinel Node (Hot, Blue, Hot & Blue) that Determines its Significance in Lymphatic Mapping." Residents forum presentaion by Dr. Arlene Chua, Southeastern Surgical Congress, February 16, 1999.

"Filtered vs. Unfiltered Technetium Sulfur Colloid in Lymphatic Mapping in a Pig Model." Accepted for oral presentation to the Society of Surgical Oncology. San Diego, March 1998.

"The Gamma Probe & Isosulfan are Complementary in Accurate Lymphatic Mapping for melanoma." Poster presentation to the Association of Academic Surgery. Dallas, Texas. November 1997.

"Sentinel Node Biopsy for Breast Cancer." North Carolina Surgical Society. Greenbriar, West Virginia. September 1997.

"Cryosurgical Treatment of Liver Lesions." North Carolina Surgical Society. Bermuda. September 1994.

"Management and Prognosis of Pulmonary Melanoma." American Society of Thoracic Surgery. New York, New York. April 1994.

"Breast Conserving Surgery for Mammographically Detected versus Palpable Invasive Carcinomas." American Radium Society. Aruba. April 1993.

"Long-Term Survivors of Melanoma Metastatic to Distant Sites." Society of Surgical Oncology. Los Angeles, California. March 1993.

"Effect of Surgical Margins and Radiation Dose on Local Recurrence after Breast - Conserving Surgery for Invasive Carcinoma", Society of Surgical Oncology. Los Angeles, California. March 1993.

"Planned Segmentectomy: A Necessity in the Management of Breast Carcinoma." Pacific Coast Surgical Association. Scottsdale, Arizona. February 1993.

"Small Bowel Subserosa as a Superior Site for Fetal Pancreas Transplantation Compared to the Renal Subcapsule Site." Association of Academic Surgery. Houston, Texas. November 1990.

"Fetal Liver and Pancreas Transplanted as a Composite Improves Islet Graft Function." XIII International Congress of the Transplantation Society. San Francisco, California. August 1990.

"Beneficial Effects of Fetal Liver Tissue on Fetal Pancreas Transplants." Central Surgical Association. Chicago, Illinois. March, 1990.

"Effects of Local Delivery of Cyclosporine on Transplanted Islets." The Society of University Surgeons Residents' Program. Los Angeles, California. February, 1990.

"Topographical Variations of Cartilage." Annual Meeting of Orthopedic Research. Rockefeller University, New York, New York. April, 1982.

Invited Presentations

"Sentinel Node Sampling for Breast Cancer." Grand Rounds speaker, Sentara Virginia Beach General Hospital, Virginia Beach, VA. November 16, 1999

"Innovations in Breast Cancer: A Diagnostic and Treatment Overview." Living Beyond Breast Cancer Rex Classic Annual Survivors Program. October 29, 1998

"Quest for the Metastatic Cell." Grand Rounds speaker. Macon, Georgia. October 22, 1998

"Surgical Oncology: Melanoma, Breast Cancer and Rectal Cancer." Invited Faculty for Saltzburg Seminars for Eastern European Surgeons. Salzburg, Austria. February 1998.

"Sentinel Node Biopsy and the Management of Malignancies" Sun Yat Sen University of Medical Sciences, Guongzou, China. October 1997.

"Sentinel Node Biopsy and the Management of Malignancies" Guangdong Cardiovascular Institute, Guongzou, China. October 1997.

"Breast Surgery" Beaufort County Hospital, Beaufort, N.C. September 1997.

"Sentinel Node Biopsy for the Management of Breast Cancer" Albemarle Hospital, Elizabeth City, North Carolina. April 1997.

"Surgical Oncology: Melanoma, Breast Cancer and Rectal Cancer." Invited Faculty for Saltzburg Seminars for Eastern European Surgeons. Salzburg, Austria. March 1997.

"Management of the Axilla - Do we need a different approach. " Advances in Breast Cancer, Greenville, North Carolina, 1996.

"The Changing Management of Melanoma" Down East Dermatology Day, Greenville, North Carolina.1996.

Grant Activity

"*Development of Compact Beta and Gamma Detectors for Biomedical Applications*" National Science Foundation, $200,000. (9/98-9/02) Funded 1998. Dr. Lorraine Tafra (Co-Investigator), Dr. Cynthia Keppel, (Principal Investigator).

"*A new technique for Diagnosis of Breast Cancer with a CW Converging Laser Beam*" U.S. Army Medical Research and Material Command, $286,496. (5/15/99-5/15/02) Submitted July 1, 1998. Dr. Lorraine Tafra (Co-Investigator), Dr. Xin-Hua Hu, (Principal Investigator).

"*Molecular Biology Studies of Sentinel Nodes*" Department of North Carolina Ladies Auxiliary to V.F.W. , $5,550. Received May, 1997. Dr. Lorraine Tafra (Principal Investigator).

"*Culturally Based Intervention for Breast Cancer in Rural African Americans*" Department of Defense Breast Cancer Program, $910,125. Dr. Donald Lannin (Principal Investigator), Dr. Lorraine Tafra (Co-Investigator) Funded 1996.

"Accuracy and Significance of Polymerase Chain Reaction Detection of Sentinel Node Metastases in Breast Cancer Patients." ECU School of Medicine Faculty Research Grant. #2-65507. $16,931. (1996-1997) Dr. Lorraine Tafra (Principle Investigator).

"Accuracy and Significance of Polymerase Chain Reaction Detection of Sentinel Node Metastases in Breast Cancer Patients." ECU School of Medicine Faculty Research Grant. (1997-1998). $18,500. Dr. Lorraine Tafra (Principle Investigator).

"Accuracy and Significance of Polymerase Chain Reaction Detection of Sentinel Node Metastases in Breast Cancer Patients." U.S. Army 1997 Breast Cancer Research Program, Career Development Award. Funded 1998. $198,440. Dr. Lorraine Tafra (Principle Investigator).

"Cryosurgery for Hepatic Malignancies: A Pilot Phase II Multicenter Trial Arrow International. $50,000/yr.. (1997-02) Dr. Lorraine Tafra (Principle Investigator).

"Improved Detection of Metastases by Lymphatic Mapping" American Cancer Society Institutional Research Grant. ECU #IRG 97-149. $15,000. Funded April 1998.

"*Molecular Biology Studies of Breast Cancer Metastases* Department of North Carolina Ladies Auxiliary to V.F.W. , $6,000. To received June, 1998. Dr. Lorraine Tafra (Principal Investigator).

"*Improved Detection of Breast Cancer Metastases Using PCR and Superior Markers for Sentinel Lymph Node Analysis.*" U.S. Army 1998 Breast Cancer Research Program, Translational Preproposal accepted, May, 1998, $2,300,000. Grant submitted July 1999.

Teaching Activities

"*Lymphatic Mapping and Sentinel Node Biopsy: Indications, Techniques and Prospects"*
Course Director, May 30 1997, November 14, 1997, February 27, 1998, May 15, 1998, June 5, 1998, August 14, 1998, December 4, 1998, March 12, 1999. East Carolina University, Greenville, N.C.

"*Lymphatic Mapping and Sentinel Node Biopsy: Indications, Techniques and Prospects"*
Course Director, October 22, 1999, January 21, 2000, April 7, 2000, July 14, 2000, November 17, 2000. Breast Center of Anne Arundel Medical Center, Annapolis, aryland in Reisterstown, Maryland.

Public Relations and Media Presentations

"*Beta Probe to detect small deposits melanoma"* WITN Channel 7 & WNCT TV Channel 9. Greenville, North Carolina. October, 1998, Anchor: Anna Holloman; Anchor: Roseann Haven. Picked up nationally. ***Content:*** Report of the first use intraoperatively of probe that could detect small amounts of nonpalpable, asymtomatic melanoma and enable surgical excision.

"*Melanoma Vaccine Clinical Trial at ECU* WITN Channel 7. Greenville, North Carolina. April 30, 1998, Anchor: Anna Holloman. ***Content:*** Review of the melanoma vaccine protocol.

"*Medical Miracle: Cryosurgery for Hepatic Malignancies"* WNCT TV Channel 9; Greenville, North Carolina. April 30, 1998, Anchor: Roseann Haven. ***Content:*** Review of cryosurgery for hepatic malignancy experience at ECU and interview with patient.

"*Melanoma vaccine: new therapy for melanoma patients"* WNBC-TV Charlotte. NBC national network TV. May 7 1998. ***Content:*** Feature segment on the new melanoma vaccine trial with ECU as an investigative site being conducted by Dr. Donal Morton.

"*ECU Minimally Invasive Surgical Team"* WNBC-TV Charlotte. NBC national network TV. May 30 1997. ***Content:*** Series on minimally invasive surgery being performed at ECU. Feature segment on sentinel node biopsy for breast cancer.

"*Breast Cancer Survivorship*" WNCT TV Channel 9; Greenville, North Carolina. Nov. 19, 1997, Anchor: Roseann Haven. ***Content:*** Attitudes, awareness, and reaction to a diagnosis of breast cancer.

"*Search for a simpler cancer test*" Raleigh News & Observer, Cover Story, Raleigh, North Carolina. Sept. 8 1997, Associated Press National Network feature story. ***Content:*** Sentinel node biopsy for breast cancer and the multi-center trial started at ECU currently underway.

Sentinel Node Biopsy Course" WNCT TV Channel 9; Greenville, North Carolina. Nov. 14, 1997, Anchor: Roseann Haven. ***Content:*** Description and impact of a course teaching sentinel node biopsy.

"*Small cuts big gain for surgery advance at ECT med. school*" The Herald-Sun, Durham, N.C. Nov. 19 1997. ***Content:*** Minimally invasive techniques being explored at ECU including sentinel node biopsy for malignancies.

"*Minimalist approach to surgery*" Raleigh News & Observer, Raleigh, North Carolina. November 19, 1997, ***Content:*** Minimally invasive techniques being explored at ECU including sentinel node biopsy for malignancies.

"*Team of ECU doctors wants to perfect new type of surgery*" Daily Reflector, Greenville, N.C. May 30 1997, Oct.. 12, 1997. ***Content:*** Minimally invasive techniques being explored at ECU.

"*Breast Cancer Education, Treatment Options and Survival*" WTKF Radio 107.3. "Positive Living", Host: Patricia Raskin. October 8, 1997. ***Content:*** One of a panel of 6 discussing breast cancer.

"*Breast Cancer*" WNCT-TV Channel 9. Carolina Today. July 16, 1997, ***Content:*** Discussion of breast cancer breakthroughs.

"*Breast Cancer and Sentinel Node Biopsy*" WITN Channel 7. Greenville, North Carolina. June 1997, Anchor: Anna Holloman. ***Content:*** Sentinel node biopsy for breast cancer and the multi-center trial.

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Title: Multicenter Trial of Sentinel Node Biopsy for Breast Cancer Using Both Technetium Sulfur Colloid and Isosulfan Blue Dye

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Mini-abstract

Report of the first multicenter trial using a combination technique of isosulfan blue dye and technetium sulfur colloid to locate the sentinel node in breast cancer patients. Trial included patients with prior lumpectomy, no limitation on tumor size, short and long time intervals of radiocolloid injection, and all gamma probe types. Age of the patient, surgeon's level of experience and medial tumor location were variables statistically affecting results.

Abstract

Objective: To determine the factors associated with false negative sentinel node biopsy (SNB) and sentinel node (SN) localization (success rate) in breast cancer patients enrolled in a multi-center trial using a combination technique of isosulfan blue (IB) with technetium sulfur colloid (Tc99).

Background: SNB is a diagnostic test to detect metastases in breast cancer patients. In order to test the reliability of this method a complete lymph node dissection must be performed to determine the false negative rate. Single institution series have reported excellent results although one multi-center trial reported a false negative rate as high as 29% using radioisotope alone. A multicenter trial was initiated to test combined use of Tc99 and IB.

Methods: Investigators (both private practice and academic surgeons) were recruited after attending a course on the technique of SNB. No investigator participated in a learning trial prior to entering patients. Tc99 and IB were injected into the peritumor region. The only exclusion criteria for entrance into the trial were palpable or suspicious axillary lymph nodes.

Results: Five hundred and twenty-nine patients underwent 535 sentinel node biopsy procedures for an overall success rate in finding a SN of 87% and false negative rate of 13%. The success rate increased, and the false negative rate decreased to 90% and 4.3% respectively, after investigators had performed more than 30 cases. Univariate analysis of tumor location and type, prior breast treatment (FNA biopsy, core biopsy, open biopsy or lumpectomy), Tc99 preparation (filtered versus unfiltered), injection timing interval, gamma probe used (C-trak, Neoprobe, Navigator), patient age, tumor size, extent of axillary involvement, and surgical experience, showed poorest success with older patients and inexperienced surgeons (<10 cases). Multivariate analysis also identified both age and experience as independent predictors of failure. Medial quadrant location of the tumor was the only variable found to significantly increase the false negative rate. However, with older patients, inexperienced surgeons, and patients with ≥ 5 metastatic axillary nodes the false negative rate was consistently higher.

Conclusions: This multi-center trial, from both private practice and academic institutions, is an excellent indicator of the general utility of SNB. It establishes those factors which play an important role (patient age, surgical experience, tumor location) as well as those that are irrelevant (prior surgery, tumor size, Tc99 timing). This widens the applicability of the technique as well as identifying factors that require further investigation.

Introduction

Since the description of sentinel lymph node biopsy (SNB) in the early 1990's, results for breast cancer have been reported in a number of single institution series [1-4]. These studies were promising and the sentinel node (SN) predicted the presence or absence of disease in the remaining axillary lymph nodes in the majority of patients. Techniques varied in these trials using isosulfan blue (IB) alone, technetium sulfur colloid (Tc99) alone, or both combined. The only multi-center trial validating this new surgical technique used Tc99 alone [5]. Other series involving large number of patients using IB and Tc99 combined, have been criticized for either failing to report an accurate false negative rate [6], or not permitting calculation of this rate by enrolling patients that did not undergo complete lymph node [4].

The impetus to perform SNB alone is great, as there is no data supporting that patients without axillary metastases will benefit from the potentially morbid procedure of axillary dissection. Unfortunately, as SNB moves to replacing axillary node dissection, the opportunity to obtain accurate data on the false negative rate under a variety of conditions is lost. The studies to date have tightly restricted the conditions under which lymphatic mapping is performed, i.e. size of tumor, timing of Tc99, and prior breast surgery. There is little data, however, to support the premise that these factors actually affect the diagnostic accuracy of the technique.

Before moving to performing SNB alone as standard of care for breast cancer patients it will be important to determine those patients that can benefit as well as those who may be ill served by the technique. Patients must undergo complete axillary node dissection to determine the false negative rate under a variety of conditions in order to determine which patients are appropriate candidates for SNB. The purpose of this trial was to determine those factors that influence lymphatic mapping in a multicenter trial by performing SNB followed by complete lymph node dissection.

Methods

Investigative Sites

A multicenter trial was conducted from February 1997 through May 1999 and enrolled patients from surgical investigators that participated in a formal lymphatic mapping and SNB

course. The course included hands-on experience in a porcine model that has previously been described [7]. The principal investigator visited most investigative sites at least once. IRB approval was obtained from all investigative sites and all patients signed informed consent. Investigative sites included both private practice and academic centers.

Technique of SNB

The technique of SNB included a peritumoral injection with 2-5 cc IB (American Regent Laboratories, Inc., Shirley NY) as well as a peritumoral injection of 1mCi (37MBq) of Tc99 (CIS-US, Bedford MA). The Tc99, in a volume of 4 cc was injected at 12, 3, 6 and 9 O'clock positions around the tumor, biopsy or lumpectomy cavity. Each investigative site had a choice of using filtered or unfiltered Tc99. Filtered Tc99 was obtained by passing radiocolloid over a 0.2μm filter. Only the timing of IB injection was restricted and it was injected immediately prior to surgery. Intra-operative use of the gamma probe was required on all patients to aid in the identification of the SN. A SN was defined as any node that was blue, both blue and hot (with hot defined as an ex vivo count equal to or greater than 10 times a background count), or hot only node, and the location of all SNs in vivo was recorded. The background count was taken off the lower extremity or abdomen. Gamma probe counts were obtained on the axillary dissection specimen, all surrounding nodal basins, the primary injection site and the SNs in vivo and ex vivo. In most cases the SN was harvested prior to performing the definitive surgery on the breast. A standard level I and II lymph node dissection was performed following SN excision.

Patients

Exclusion criteria were clinically suspicious or positive axillary nodes, pregnancy, and extensive cardiac, pulmonary or renal disease. Data collected intraoperatively included the type of gamma probe used (C-track {Carewise, Morgan Hill, California}, Neoprobe {Neoprobe Corp., Dublin, Ohio} and Navigator {U.S. Surgical, Bedford, Ohio}), technique of diagnosis (including patients undergoing prior lumpectomy, open biopsy, FNA biopsy or core biopsy), tumor size, time of Tc99 injection, time of SN harvest, and use of filtered vs. unfiltered Tc99.

Radiation Safety

A radiation safety officer participated in the initial start-up of the protocol at each site. Radiation exposure was monitored in the radiology suite, the operating room, and the pathology

laboratory. The primary tumor or site of Tc99 injection was kept for 24-48 hours prior to pathological analysis to allow for sufficient decay.

Pathology

All SNs were serially sectioned, and every other section was submitted for additional laboratory studies. Each section submitted to pathology was analyzed using multiple sections. The majority of H & E negative SNs were analyzed by immunohistochemistry with a cytokeratin cocktail (Cytokeratin AE1:3, Boehringer Mannheim Corp., Indianapolis, IN).

Statistics

Fisher's exact test for nominal variables was used to compare success rates and false negative rates according to surgeon experience, patient age, tumor size, location of the primary tumor, histological tumor type, type of previous biopsy, filtered or unfiltered Tc99, time from injection to surgery, number of positive nodes, number of SNs found, type of gamma probe used, and ex vivo SN counts. Any of these variables with a p-value <0.25 were used to develop a multivariate logistic regression model to predict failure to find a SN. The Hosmer-Lemeshow goodness-of-fit criterion was used to determine the adequacy of the model. The success rate (also referred to as the identification rate) in this trial is defined as the ability to successfully identify a SN on a per procedure basis (a small number of patients underwent bilateral SNB). The false negative rate is defined as the number of procedures with a negative SN (and a positive non-SN) divided by the procedures with positive axillary lymph nodes. A one-way analysis of variance was used to compare the mean number of nodes found and mean ex vivo counts between injection intervals, type of previous biopsy, and filtered or unfiltered Tc99. Fisher's exact test was used to assess the relationship between the number of SNs found and the number of those nodes that were positive. The SPSS 8.0 statistical software was used for the statistical analysis. All reported p-values for Fisher's exact test were two-tailed.

Results

A total of 529 patients enrolled in the study. The mean (+/- SD) number of lymph nodes obtained during axillary dissection was 13 (+/-6). Forty-eight surgeons participated in the trial and contributed a median of 18 patients. Characteristics of the study population are summarized in Table 1. The majority of patients had upper outer quadrant lesions, pathology consistent with infiltrating ductal carcinoma, and underwent lumpectomy (341, 70%) compared to mastectomy

(146, 30%). The mean age of the entire group was 57 and ranged from 26 to 89 years old. Six patients underwent bilateral SNB for bilateral breast cancer. Sixteen percent of patients were found to have an internal mammary basin count greater than background but SNB was not performed although the option was available to the investigators. Table 2 presents the status of the lymph nodes in all sentinel node procedures where a SN was found. Of the 535 sentinel node procedures a sentinel node was identified in 466 for an identification rate of 87%. The false negative rate for the group was 13%. The accuracy of the SNs to detect metastatic disease was 96% and the negative predictive value was 95%. Forty-six patients were excluded from the false negative analysis for lack of a complete lymph node dissection, but included in analysis of success (or identification) rates (Table 3, 4, 6, 7 and 8).

Factors associated with not finding the SN and false negative SNs:

A univariate analysis which examined eight variables (Table 3) found that only patient age ($\geq$50 years) and surgical experience (performance of < 10 cases) significantly affected the success rate of finding the SN. Success rates were lower in patients that had a prior lumpectomy, $\geq$ 5 positive lymph nodes on axillary dissection, lesions located in the medial quadrant, or Tc99 timing intervals less than 30 minutes but these did not reach statistical significance. A multivariate analysis showed surgical experience and age were independent predictors of failed SNB (Table 4).

The number of false negatives in this series was small and totaled 18. A univariate analysis examined nine variables to determine their influence on the false negative rate (Table 5). Although the false negative rate was high among inexperienced surgeons and procedures yielding only one SN, only the location of a tumor in the inner quadrant reached statistical significance.

Fourteen patients in the trial had preoperative chemotherapy. There were no false negatives and a SN was identified in each patient. Lymphovascular invasion data was available in 284 patients. The success rate was 80% in the 50 patients with this tumor characteristic compared to 87% in patients whose tumors lacked lymphovascular invasion (NS).

Figure 1 depicts false negative rates and success rates for increasing patient age showing the success rate steadily decreases and the false negative varies widely over these age groups. With increasing surgical experience, the false negative rate decreased and the success rate increased (Figure 2).

Number of SNs

A total of 1055 SNs were found with a mean of 2.2 +/- 1.4 per patient. The majority of patients had either 1 (41%) or 2 (30%) SNs found with 29% having 3 or more. There was an increased chance (p=0.025) of having a positive SN if more than one SN was found (Table 6). As stated above this translated into a lower false negative rate (Table 5). The number of SNs found had no bearing on the overall chance of having positive axillary nodes or on the number of positive nodes (data not shown).

Factors affecting the number of SNs obtained were examined (Table 7). Older patients were found to have significantly fewer SNs. With increasing injection interval the mean number of SNs obtained increased (p=0.01).

Characteristics of the SNs were available for 849 nodes. Sixty-four (7%) of these were blue only (radiation activity less than 10X background). Of the remaining SNs, 57% were both hot and blue and 36% were hot only.

Gamma Probe Counts

The pattern of uptake of Tc99 by the SN differed depending on whether or not the Tc99 was filtered (Table 8). Since many patients had more than one SN and counts varied on each SN, calculations were made on both the hottest node (mean hottest node counts) and all the SNs (mean counts all SNs). In the unfiltered group, counts on the SNs increased steadily with increasing time after Tc99 injection. In the filtered group, however, the counts increased rapidly between 21 to 60 minutes and then decreased.

The positive SN was the hottest node in 77% of those patients with a positive SN in whom more than 1 SN was found. In 23% of the cases, however, the positive node would have been missed if only the hottest node had been removed.

Discussion

Since the publication of the first series on SNB for breast cancer [8], there has been overwhelming enthusiasm for development of this technique. SNB represents a major advance because it is not only minimally invasive, but despite less surgery, improves the accuracy of pathological staging [9]. This study was performed to delineate those factors that are responsible for a false negative SN or failing to identify a SN when a combined Tc99 and IB technique is

used. As the investigators were from a variety of centers and consisted of general surgeons as well as breast specialists it is an excellent indicator of the general utility of the technique. The results indicate that caution must be used when performing SNB on older patients and patients with medial quadrant lesions. In addition, it is clear from this data that the level of surgical expertise with the technique is crucial. Performance of at least 30 cases is required to insure the success rate is over 90% and the false negative rate drops below 5%. The data do not support the conclusion that patients who have undergone prior breast surgery or have a larger tumor are ineligible for the procedure. These results, therefore, broaden the applicability of the technique to patients that have until now been excluded in most series.

It remains unclear if the technique of using both IB and Tc99 is superior to either agent used alone. The single published multicenter trial using Tc99 alone [5] included only experienced surgeons and achieved a false negative rate that differed by 2% (11 vs. 13%) from this trial with relatively inexperienced surgeons. The combination technique may make it easier to find a SN and reduce dissection time, but proof of this would require further study. The results of blue dye alone in experienced hands has been very good [3,10]. The concern with use of this agent alone, however, is that a second SN may be missed whereas a second radioactive SN could be detected with the gamma probe. In addition, the finding of a "cold axilla" is reassuring to the surgeon that all SNs have been removed. The data from this trial suggest that surgeons should not stop after finding just one SN but should search thoroughly to be certain there are not more. This is especially appropriate as first, the mean number of SNs obtained in this trial was 2, and second, the false negative rate in patients yielding 1 SN was 20.8% compared to 9.2% ($p=0.07$) when a second SN was searched for and found.

In this large clinical trial with a relatively high false negative rate, we found that location of tumors in the medial quadrant increased the false negative rate. This finding is somewhat baffling and with no obvious physiological or anatomic explanation it possibly represents a statistical error in the small population of patients with false negative SNs. High false negative rates were found in patients in whom only one SN was found and with inexperienced surgeons, but these did not reach statistical significance. As the actual number of patients with a false negative SN is small, an even larger series of patients will be required to definitively determine those factors that can predict a false negative result.

A variety of injection techniques have been described [2,11-13] and it is not clear whether one is optimal. These techniques include a peritumoral injection, subdermal injection, as well as injection of Sappey's plexus. As the area of the peritumor injection increases, (i.e. compare the

injection area of a small biopsy to a lumpectomy), the number of SNs obtained could potentially increase. If the injection technique leads to finding a large number of SNs, this ultimately dilutes the SN concept. Some studies have found a lower success rate in patients that have undergone a prior breast biopsy [5,14,15] and others have not [3,16]. We found that removing breast tissue for either a breast biopsy or a therapeutic lumpectomy did not statistically alter either the false negative rate or the success rate. These results support the hypothesis that perhaps the entire breast drains to only one or two SNs. This is further supported by not finding a greater number of SNs in those patients who had a prior biopsy or lumpectomy (Table 7). If confirmed in larger series, the data would suggest that *precise* injection of IB or Tc99 at the tumor site might not be necessary.

In this study, the affect of increasing age of the patient was very significant. Not only are fewer SNs found, they are found less frequently. Krag et al [5] reported a similar finding and hypothesized that this is secondary to the progressive replacement of the parenchyma of the lymph nodes in older patients by fat. It is also possible that with increasing age of the patient, the breast becomes progressively replaced by fat, and the lymphatic vessel density decreases. Lymphatic uptake of the dye and Tc99 may become more sluggish resulting in less concentration of the Tc99 or IB in the SN, making SN identification more difficult. Further research will be needed to formulate better techniques in the elderly to guarantee a high success rate in this age group.

It has been hypothesized that as the tumor volume in the axilla increases, the success rate of SNB decreases and the false negative rate increases. The biological rationale behind this hypothesis is that lymphatics get progressively infiltrated with tumor cells and do not allow the passage of dyes or radionucleotides. Our success rate was lower when patients had $\geq$ 5 involved axillary nodes, but this did not reach statistical significance. The small number of patients with greater than five positive axillary nodes could account for this. The success rate was also lower in patients with tumor lymphovascular invasion (80% versus 87%). Until larger series confirm extent of axillary involvement as an important factor, it is probably wise in patients undergoing SNB alone, especially in whom a clinical examination is difficult (i.e. obese patients), *that intra-axillary palpation through the SNB incision be performed.* Any suspicious nodes should be biopsied. The possibility of obtaining a false negative SN in a patient with bulky disease in the axilla is very concerning, since that patient would be at high risk of systemic failure, and potentially would be denied chemotherapy based on the false results of the SN. The patient would also be denied any possible therapeutic benefit of removing malignant lymph nodes.

We have previously shown in an animal model of lymphatic mapping that use of filtered versus unfiltered Tc99 resulted in finding more SNs [7]. This was not the case in this trial. Confounding factors making this comparison difficult are different injection techniques used (peritumoral vs. subdermal) and a probable inherent difference between the swine and human lymphatic systems. We did find, however, less variation in gamma probe counts when the unfiltered preparation was used. As neither preparation affected the false negative rate or success rate, it is unlikely to be an important factor in the technique of lymphatic mapping with experienced surgeons. It may, however, be an important factor in decreasing the learning curve of SN identification. The hotter the SN the easier it is to detect at the skin level. To obtain this using filtered takes a short injection time interval (30–60 minutes) whereas a longer injection interval may be more optimal for use of unfiltered Tc99 (60-240 minutes).

Tumors in the medial quadrant have posed a number of concerns and questions with the introduction of lymphatic mapping for breast cancer. A significant number of patients with a medial quadrant lesion will drain to the internal mammary nodes and present with metastases in this lymphatic chain [17-19]. Data from early randomized trials showed removal of these lymph nodes could increase survival (at the cost of very high morbidity) [20]. SNB has the potential to identify the group of patients at risk for these metastases by identifying an internal mammary SN. Dissection of internal mammary SNs has been extensively studied in one series using 10 mCi of radiocolloid with excellent results [16]. Seventy patients were reported and 34% of patients on lymphoscintigraphy were found to have an internal mammary SN (24). These were successfully dissected in 62% (15/24) patients and positive in 33% (5/15). Two patients did unfortunately suffer a pneumothorax. In our trial a much lower rate of 16% of patients was found to have a "hot spot" at the internal mammary basin with counts 10X a background count but dissection was not performed. The difference in rate could be attributed to the lower dose of Tc99 used in this clinical trial. It is difficult to determine whether these hot spots represent true internal mammary SNs or "shine-through" from a hot primary close to this chain of lymph nodes. The incidence of internal mammary metastases is low and therefore a clinical trial to evaluate the therapeutic benefit of either positive internal mammary SN removal or treatment of positive internal mammary nodes with radiation, would be difficult. For the first time, however, it is possible with minimal morbidity, to tailor treatment for those patients at risk for metastases in this chain of lymph nodes.

In summary, the combined use of IB and Tc99 can identify SNs accurately but requires surgical expertise to reach what has been viewed as an acceptable level of success and false negative rate (>85%, <5% respectively)[21]. These data suggest caution is required in older

patients and in patients with medial quadrant lesions. This study, however, also demonstrates the usefulness of the SNB even in patients with larger tumors and those who have undergone prior breast surgery. Other factors beyond the surgeons' control, such as large axillary tumor burden, may contribute to SNB failure. Progression to performing SN alone by the surgical community will need to be accompanied by a thorough intra-axillary examination to attempt to decrease the false negative rate. Although it is not well established what false negative rate, if any, will translate into decreased survival, it must be remembered that the technique will spare more than half of breast cancer patients an axillary node dissection that carries significant morbidity and provides no benefit when the lymph nodes are negative.

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Legends

Figure 2: Effect of surgical experience on SNB.

This figure demonstrates the important affects (decreased false negative rate and increased success rate) of increasing experience of the surgeon.

Legends

Figure I: Effect of patient age on SNB.

This figure demonstrates the adverse affects (decreased success rate) of increasing age of the patient.

Table 1. Patient Characteristics.

Characteristic	Groups	# Pts. (%)
Tumor Type	Infiltrating ductal	393 (74%)
	Infiltrating lobular	36 (7%)
	Other	92 (16%)
	Unknown	14 (3%)
Tumor Location	Upper outer quadrant	244 (46%)
	Upper inner quadrant	66 (12%)
	Lower outer quadrant	45 (8%)
	Lower inner quadrant	39 (7%)
	Central	56 (11%)
	Other	70 (13%)
	Unknown	15 (3%)
Tumor Size	<2 cm	289 (54%)
	2-5 cm	192 (36%)
	>5 cm	16 (3%)
	Multifocal	38 (7%)
Prior Surgical Procedure	Tumor Intact (FNA or Core)	334 (62%)
	Open Biopsy	165 (31%)
	Lumpectomy	34 (6%)
	Unknown	2 (<1)
Tc-99 Preparation	Filtered	211 (39%)
	Unfiltered	321 (60%)
	Unknown	3 (1%)
Gamma Probe Used	C-trak	257 (48%)
	Neoprobe	204 (38%)
	Navigator	66 (12%)
	Unknown	8 (2%)

Table 2. Number of Procedures with Metastases to SNs and Axillary Nodes.

	Axillary Nodes		
Sentinel Nodes	Positive	Negative	Total
Positive	122		122
Negative	18	326	344
Total	140	326	**466**

TABLE 3. SN Identification (Success) Rate

	Success %	P
Age		
< 50	95.0	
≥ 50	84.6	0.001
Prior Surgery		
FNA/Core Biopsy	86.8	
Open Biopsy	90.6	
Lumpectomy	81.8	0.17
Tc99 Status		
Filtered	87.3	
Unfiltered	88.5	0.68
Total Positive Nodes		
< 5	88.0	
≥ 5	75.0	0.17
Injection Time		
< 30 Minutes	80.8	
≥ 30 Minutes	88.8	0.06
Tumor Size		
< 2 cm	86.4	
2-5 cm	89.0	
> 5 cm	87.5	0.72
Tumor Location		
Inner	83.3	
Outer	89.4	
Central	94.4	0.10
Surgeon Experience		
< 10 Cases	82.1	
≥ 10 Cases	91.8	0.002

Table 4. Multivariate Analysis of Factors Affecting Decreased Success in Finding a SN.

Variable		Failure Rate	Odds Ratio	Odds Ratio (95% CI)
Age				
	< 50	5.1	1.00	
	≥ 50	15.4	3.46	1.31 – 9.22
Experience				
	> 10 Cases	8.2	1.00	
	≤ 10 Cases	17.9	2.72	1.30 – 5.71

TABLE 5. Factors Affecting False Negative Rate.

	FN Rate %	P
Age		
< 50	10.3	
≥ 50	15.1	0.60
Prior Surgery		
FNA/Core Biopsy	14.3	
Open Biopsy	11.4	
Lumpectomy	11.1	0.90
Tc99-m Status		
Filtered	10.9	
Unfiltered	15.0	0.61
Total Positive Nodes		
< 5	12.2	
≥ 5	25.0	0.20
Injection Time		
< 30 Minutes	17.2	
≥ 30 Minutes	12.2	0.54
Tumor Size		
< 2 cm	17.3	
2-5 cm	11.9	
> 5 cm	0.0	0.43
Tumor Location		
Inner	30.0	
Outer	6.9	
Central	10.0	0.02
Surgeon Experience		
< 30 Cases	15.5	
≥ 30 Cases	4.0	0.19
Number of SN's		
1 SN	20.8	
≥ 2 SN	9.2	0.07

Table 6. Relationship Between Number of SNs Obtained and Rate of SN Positivity.

# SNs Found	#Pts.	# Pts. with (+) SN	% SN Positivity
1	190	39	20.5
≥ 2	276	83	30.0
P Value			.025

Table 7. Factors Affecting Number of SNs Found

Characteristic	Variable	Mean # of SN's Found	P Value
Tumor Location	UOQ + LOQ (Outer)	2.08	
	UIQ + LIQ (Inner)	1.71	0.09
	Central	2.32	
Prior Surgical Procedure	Tumor Intact (FNA or Core)	1.96	
	Open Biopsy	2.08	0.34
	Lumpectomy	1.59	
Tc99 Preparation	Filtered	1.96	
	Unfiltered	2.00	0.79
Gamma Probe Used	C-trak	1.93	
	Neoprobe	2.00	
	Navigator	2.18	0.59
Age at Diagnosis	<50 yr.	2.51	
	≥50 yr.	1.74	0.001
Injection Interval	< 20 Minutes	1.57	
	21-60	1.61	
	61-240	2.21	0.01
	> 240	1.89	

Table 8. Relationship Between Timing of Tc99 and Ex Vivo SN Counts Using Filtered and Unfiltered Preparations

FILTERED

Timing of Tc99 (min.)	Mean Hottest Node Counts	Mean Counts all SN's
< 20	2938	2609
21-60	24558	11563
61-240	4629	3583
> 240	1863	1519

UNFILTERED

Timing of Tc99 (min.)	Mean Hottest Node Counts	Mean Counts all SN's
< 20	2683	1816
21-60	3332	1937
61-240	9178	5142
> 240	8683	5886

SCIENTIFIC PAPERS

Credentialing Issues with Sentinel Lymph Node Staging for Breast Cancer

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Sentinel lymphadenectomy (SL) is a minimally invasive approach for staging patients with breast cancer. SL, when performed in lieu of axillary dissection, is associated with less morbidity and is potentially more cost effective and more accurate than the historical axillary dissection in the detection of regional nodal metastasis. The credentialing and privileging of SL, as with any surgical procedure, is by the policies of the local hospital or institution. The suggested credentialing criteria for local hospitals has been an area of controversy. Herein the authors outline the credentialing controversy and suggest criteria for the implementation of sentinel lymph node staging for breast cancer. ***Am J Surg.*** **2000;180:000–000. © 2000 by Excerpta Medica, Inc.**

The issue of credentialing for any new surgical procedure is often complex and sentinel node biopsy is no exception. The questions that must be answered include (1) how many procedures should a surgeon perform before he or she achieves an adequate level of expertise, (2) how low an identification rate is acceptable without denying too many patients the opportunity to benefit from a noninvasive procedure, and possibly of most importance, (3) how high a false-negative rate is acceptable without impacting on the morbidity and mortality of our breast cancer patients? The data on sentinel node biopsy is accumulating quickly. As with any new surgical procedure, each publication brings to light new aspects of the procedure that have previously been unrecognized or ignored. The emerging consensus is clear. Sentinel lymph node biopsy has been validated as an accurate staging technique for patients with stage I and II breast cancer by multiple series reported from single institutions and the recent report from at least two multicenter trials at the annual meeting of the American Society of Breast Surgeons.

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DEFINING THE PARAMETERS OF SUCCESS

There are two key parameters of successful sentinel lymph biopsy: the sentinel lymph node identification rate and the false-negative rate. The unfortunate publication of work that confuses the identification rate with the false-negative rate has unnecessarily confounded the credentialing issue. A number of studies have documented factors that influence and do not influence the identification rate, but erroneously called this the "success rate," further confusing the efforts in credentialing.[1]

Although it is important to determine those factors that affect the identification rate, it is the false-negative rate that may adversely impact a patient's prognosis. There are two inescapable conclusions when one examines all of the data related to sentinel lymph node biopsy for breast cancer. First, sentinel lymph node biopsy can accurately determine the nodal status for breast cancer and can replace axillary lymph node dissection as the staging procedure of choice. Secondly, sentinel lymph node biopsy can be performed badly with unacceptably high false-negative rates. Implementation of a successful sentinel lymph node biopsy program requires extensive interdisciplinary coordination and planning, and should, in the view of the authors, include an initial validating series of sentinel node biopsy with simultaneous axillary dissection to ensure a safe transition from axillary dissection.

Sentinel lymph node biopsy is a diagnostic test to stage the axillary lymph nodes. A comparison of complete axillary lymph node dissection as a staging procedure is given in Table. As with any diagnostic test the critical issues are T1
the sensitivity, specificity, positive and negative predictive values, overall accuracy and the false-negative rate. What is an acceptable false-negative rate? We know that we already accept a 2% to 3% false-negative rate by only performing level I and II axillary lymph node dissection. This is because level III contains a 2% to 3% incidence of "skip metastases." The American Society of Breast Surgeons has previously issued a consensus statement that suggests that the false-negative rate for breast cancer sentinel lymph node biopsy should be 5% or less.[2]

It is also important to recognize that sentinel lymph node biopsy is more accurate than axillary dissection. This is because sentinel lymph node biopsy focuses the pathologists attention on the node or nodes most likely to contain metastatic disease. Therefore, instead of performing one histological section through the center of 20 lymph nodes from the axillary dissection specimen, the pathologist can

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TABLE
Comparison of Sentinel Lymph Node Biopsy and Complete Axillary Node Dissection as Staging Procedure

	Experienced SNB Alone	Inexperienced SNB Alone	Complete LND Without SNB
Highest false-negative rate	3%–5%	28%	10%–29% (not analyzing LNs thoroughly as in SNB)
Lowest false-negative rate	0%	? 28%	2%–3% (level III LNs not dissected)
Impact of missed metastases on local axillary recurrence	3%– 5% (no lymph node dissection performed)	5%–15%?	2%–3% (level III LNs not dissected)
Morbidity	1%–2%	? Probably higher than 5%	10%–90% from lymphedema to dysesthesias
Survival	?	?	?

SNB = sentinel node biopsy; LND = lymph node dissection.

perform 20 sections with immunohistochemical staining for 1 or 2 sentinel nodes. Giuliano et al[3] have documented prospectively this improvement in staging.

THE COMPARISON WITH MELANOMA

In order to come to appropriate conclusions on the issue of credentialing, it is worthwhile to look back at the history of sentinel node biopsy and its use with melanoma compared to breast cancer. Initial single institutions series for melanoma documented a high success rate and a low false-negative rate in those patients who underwent sentinel node biopsy and complete lymph node dissection. As has been frequently stated already, a false-negative rate cannot be determined without a sentinel node biopsy being accompanied by a complete lymph node dissection. The success rates for the initial series of melanoma ranged from 80% to 100% and the false-negative rates ranged between 0% and 5%.[4–7] In reviewing the initial series, however, for sentinel node performed for breast cancer, the identification rate for sentinel node biopsy is significantly lower and the false-negative rate is significantly higher.[8–10] The first large series of sentinel node biopsy for breast cancer by Giuliano et al[8] reported a success rate of only 65% with a false-negative rate of 12%. By comparing the results of these initial series it is clear that it is more difficult to perform sentinel node biopsy correctly for breast cancer than for melanoma.

In addition, the impact of false-negative sentinel nodes is greater for breast disease than it is for melanoma. The rate of metastatic disease in axillary lymph nodes from breast cancer is almost twice that for melanoma (approximately 40% versus 20%). It would follow, then, that the impact of missing metastatic disease is also twice that for melanoma. If you assume a 5% false-negative rate for 1,000 melanoma patients, you will leave behind positive metastatic disease in 10 patients, in approximately 200 patients with metastatic disease. With a false-negative rate as high as 15% in a similar group of 1,000 patients with breast cancer, approximately 60 patients of 400 with positive nodes will be left with undiagnosed metastatic disease in their axilla. Although it can be argued that the false-negative rate of a complete axillary node dissection (without any sentinel node biopsy) can be as high as 9% with pathologists missing disease on their cursory examination; however, in all of these patients, the axillary nodes are removed.[11–15] In patients undergoing sentinel node biopsy, missed metastatic disease remains in the axilla. It remains unclear if missed metastatic disease in the axillary nodes would result in a decreased survival rate in these patients. However, an additional problem would be that these patients are also denied potentially curative chemotherapy.

Lymphatic drainage from a skin melanoma is relatively straightforward, but not so for the breast or accompanying breast disease. There are potentially influencing factors that have little impact on sentinel node biopsy for melanoma but may impact on performance of the same technique for breast disease. These include size of tumor, location of the tumor, depth of the tumor in the breast, prior excision of the breast tumor or performance of a breast lumpectomy, technetium injection time intervals and technetium preparation. In order for sentinel node biopsy to be widely accepted for breast disease and replace axillary node dissection as an accurate diagnostic tool, it is important to determine whether these factors, in fact, actually influence the accuracy of the procedure. Initial single institution series were performed to validate the concept of the sentinel node predicting the presence or absence of disease in the remaining axillary nodes. By very nature of these series, the patients were highly selected and many of these factors were not assessed. As sentinel node biopsy has gained popularity, progression to performance of sentinel node alone is growing quickly and the ability to obtain false-negative rates in controlled studies looking at these factors is rapidly decreasing.

REGISTRIES OF MULTI-INSTITUTIONAL EXPERIENCE

It is important to realize that not all questions in medicine require prospective randomized studies. In fact, the establishment of the validity of a diagnostic test, like sentinel lymph node biopsy for staging, requires the determination of the node identification rate, sensitivity, specificity, positive and negative predictive values, overall accuracy and false-negative rate. These parameters do not require randomization; they are, rather, accomplished by the prospective data collection from a large series of patients. It is also important that these values be established from a broad range of clinical practice and hospital envi-

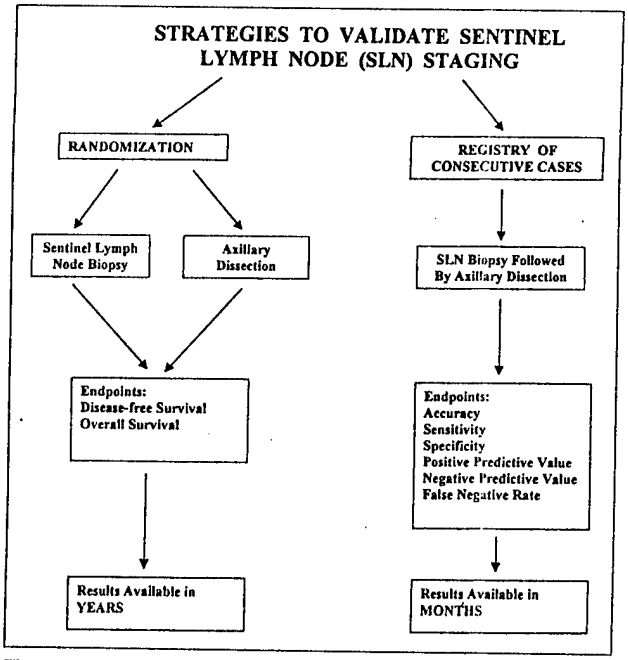

Figure 1. Strategies to validate sentinel lymph node (SLN) staging.

ronments. Compared with single institution series, multicenter trials remain superior in determining the accuracy of a new diagnostic test such as sentinel node biopsy (**Figure 1**).

Only one multicenter trial has been published and it was unfortunately restricted to use of Tc 99 alone.[16] Although the overall false-negative rate was 11%, the highest reached 29%. All surgeons had a number of practice procedures prior to enrolling patients, therefore, a ~~fine~~ learning curve could not be determined. The study was meticulously done, but was tightly restricted in terms of prior surgical history, tumor size, probe type used, Tc 99 preparation and Tc 99 timing internal. It did reveal a significantly decreased identification rate in older patients, that has been confirmed with the two multicenter trials reported below.

In 1996, the East Carolina University Medical Center and Anne Arundel Breast Center Sentinel Node Breast Project was formed in order to quantitate these parameters.[17] The Multicenter Trial obtained Department of Defense funding and was IRB approved at all investigative sites. No surgeon in this study participated in a learning trial and therefore, the data accumulated were able to determine learning curves for both the identification rate and the false-negative rate. Patients with any size tumor, and any form of prior breast surgery, were included and most sentinel nodes were evaluated by serial sectioning and immuno-staining with every other section going for polymerase chain reaction detection of metastatic disease. All patients underwent a peritumoral injection of technetium sulfur colloid and 4 cc of isosulfan blue dye. Following sentinel node harvest, 87% of patients then underwent a level I and II axillary node dissection and only these patients were used to determine the false-negative rates. All marketed gamma probes were used. The sentinel nodes were defined as either blue only, both blue and hot, or hot only. Factors that were analyzed to potentially influence the identification rate or the false-negative rate included age, prior breast surgery, the technetium 99 preparation used, the amount of disease in the axilla, the injection time intervals, tumor size, tumor location, surgeon's level of experience and the specific gamma probe used.

Six hundred and forty-eight patients enrolled between 1996 and January of the year 2000 with 18 academic[5] and private practice[13] sites contributing patients. A total of 20 patients had a false-negative in a group of 167 patients with positive nodes for a false-negative rate of 11.9%. The identification rate in those patients that underwent complete axillary node dissection was 87%. The identification rate was significantly higher in those patients less than 50 years of age compared with those greater than 50 years of age (96% versus 82%, $P = 0.0005$) and when the surgical experience consisted of greater than 10 prior procedures compared with less than 10 prior procedures (91% versus 81%, $P = 0.005$). In multivariate analysis, only these two factors remained statistically significant.

As only 20 patients had a false negative, determining those factors that influenced the false-negative rate was only possible through a univariate analysis. The only statistically significant factor was tumors located in the inner quadrant with a false-negative rate of 30% compared with the outer quadrants with a false-negative rate of 7% ($P = 0.02$). The false-negative rate for surgeons performing greater than 10 procedures was 4% compared with 15% for surgeons performing less than 10 surgical procedures (not significant). On the actual learning curves, the false-negative rate dropped below 5% after surgeons had performed more than 30 procedures.

Two other factors were not statistically significant but are, most probably, clinically significant. These factors include the number of sentinel nodes obtained and the total number of positive lymph nodes in the axilla. If only 1 sentinel node is obtained, the false-negative rate was 21% compared with 9% if greater or equal to 2 sentinel nodes was obtained. This underscores the importance of looking for a second sentinel node after the first has been removed. Further support for aggressively pursuing the second sentinel node is that the majority of single institution series have documented a mean number of sentinel nodes per patient to be just over 2. Equally important was a false-negative rate of 25% in those patients with a total of greater than 5 positive nodes in the axilla. It has been hypothesized that with increasing bulk of disease in the axilla, lymphatics progressively become obstructed with tumor cells and the injected technetium and isosulfan blue are redirected to false-negative sentinel nodes. The importance of performing axillary palpation to clinically determine and find any suspicious nonsentinel nodes cannot be overemphasized. In all cases, these suspicious nodes should be biopsied in addition to the sentinel nodes.

To the best of our knowledge, no other published series has documented the learning curve for surgeons in a multicenter trial, with no prior experience, in patients that have undergone a sentinel node with a complete lymph node dissection. The data presented here, coupled with early data from the University of Louisville Breast Cancer

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Figure 2. Credentialing guidelines.

Sentinel Lymph Node Study, ongoing since 1997, are unique in this regard.

The University of Louisville Breast Cancer Sentinel Lymph Node Study is a registry of a series of patients with T1-2, N0 breast cancer who underwent level I and II axillary lymph node dissection after sentinel lymph biopsy. All surgeons signed an investigator agreement in which they agree to submit consecutive cases and submit data on all patients who sign the consent form and also agree to data auditing and site visits. Unpublished data from the University of Louisville Breast Cancer Sentinel Lymph Node Study presented at the annual meeting of the American Society of Breast Surgeons found that surgeons who perform more than 20 cases had a superior sentinel lymph node identification rate compared with surgeons who performed between 1 and 20 cases. For surgeons who performed 1 to 20 cases the false-negative rate was 9.6% versus 1.3% for surgeons who had performed more than 20 cases, suggesting that 20 cases may be sufficient for adequate training of surgeons to perform sentinel lymph node biopsy in lieu of axillary dissection.

CONSENSUS STATEMENT OF THE AMERICAN SOCIETY OF BREAST SURGEONS

The first consensus statement released concerning the performance of sentinel node biopsy alone in place of axillary node dissection was from the American Society of Breast Surgeons. The statement suggested documenting experience with greater or equal to 30 cases with both sentinel node and axillary node dissection performed with an 85% identification rate and a false-negative rate less than or equal to 5%. Although the American College of Surgery did not release a consensus statement, in forming their Z0010 and Z0011 clinical trials in which surgeons must document these same credentialing criteria, they adopted these same criteria established by the American Society of Breast Surgeons. The American Society of Breast Surgeons has recently revised this statement (**Appendix**).

Q: 1 The previous credentialing guidelines (**Figure 2**), as established by the American Society of Breast Surgeons, seems straightforward and supported by the data presented earlier; however, there are a number of issues that need further clarification. These include the following: (1) How many procedures are required to gain expertise after a false-negative result? (2) Does the intradermal injection, with its higher identification rate, decrease the learning curve? (3) Does the multicenter results underestimate the false-negative rate in community practice? (4) Are there factors beyond the surgeon's experience that influence the false-negative rate (ie, bulky disease in the axilla)? (5) Does mentoring or proctoring from an experienced sentinel node surgeon speed up the learning curve? (6) Does a certain number of continuing medical education hours supplant the need for a portion or all of the required series of personal surgical experience?

MEDICARE CARE GUIDELINES—A SOURCE OF CONFUSION

Medicare has taken it upon itself to establish guidelines dictated through a number of local newsletters and reports. They promote the concept that credentialing should be performed by the facility (hospital, surgery unit, and so forth) and expect the minimum of the completion of a sentinel node course and performance of "an established number of procedures." No Medicare report has yet dictated the number of procedures that must be performed before moving to performing sentinel lymph node biopsy alone.

Although some conclude that it seems reasonable that Medicare established guidelines for credentialing, Medicare has also established guidelines for who should undergo sentinel node biopsy. These guidelines vary from state to state, but share the common characteristics of being confusing and inappropriate. An example of this confusion is the medical report in September of 1999, published in Maryland, that required the tumor size of a patient undergoing sentinel node biopsy alone to be less than or equal to 2 cm. Currently, there are no data to support that larger tumor sizes are a contraindication for sentinel node biopsy alone. In fact, the majority of single institution series have included patients with tumors greater than 2 cm. This clearly impacts negatively on patient care in denying the less invasive procedure to those patients. Another example is the exclusion of performing sentinel node biopsy on patients who have DCIS, irrespective of whether this was diagnosed by a core biopsy or an open biopsy. This clearly ignores the common scenario of an invasive lesion being found in an area of DCIS diagnosed by core biopsy. The patient who elects a mastectomy for the DCIS tumor would ultimately be denied the less invasive sentinel node biopsy as this cannot be performed in a patient who has had a mastectomy. She would subsequently require a complete axillary node dissection. Of even more concern is the lack of clarity in Medicare guidelines concerning patients who undergo sentinel node biopsy followed by axillary node dissection. These guidelines were established to avoid compensation to those surgeons learning the technique. However, this ignores the obvious scenario of a sentinel node being found positive and the patient subsequently requiring a complete axillary node dissection.

The only absolute contraindication that currently exists for sentinel node biopsy alone is a patient with clinically suspicious axillary metastases. Controversial contraindications are prior chemotherapy and patients with multifocal disease. With further study, these will most probably drop by the wayside. Steps are being taken by representatives of the American Society of Breast Surgeons to resolve these credentialing issues with Medicare. Sentinel node biopsy, as a new diagnostic surgical procedure, presents valuable data that again supports the detrimental impact the health care controlling agencies, such as Medicare and HMOs, have on patients. Leaving the decisions to proceed or not proceed with sentinel node biopsy up to the surgeon is a safer policy for our patients. Since the introduction of the new technique, these agencies have only served to slow surgical progress and innovation.

RECOMMENDATION FOR SENTINEL LYMPH NODE BIOPSY CREDENTIALING

The data currently available support the performance of 20 to 30 cases. Before moving to performing sentinel lymph node biopsy alone in place of complete lymph node dissection, there is no getting past the fact that, for some general surgeons, this may require more than 1 year of training to gain this expertise. It is a moral as well as practical concern to avoid discriminating against inexperienced surgeons while ensuring our breast cancer patients receive the best care.

There are a number of steps that can be taken to meet credentialing guidelines in an expeditious manner. (1) Assemble an enthusiastic team to include pathologists, nuclear radiologists, radiation safety officers, and surgeons. (2) A protocol should be obtained, of which there are many available, and ideally, IRB approval should be obtained. (3) Assign an experienced sentinel node surgeon, presumably with good knowledge and understanding of the literature, to review the results of the sentinel lymph node biopsy procedures for all general surgeons at any particular site. (4) Partner with a mentor to increase volume of all interested in learning the procedure. It has been suggested that residents mentored through 30 cases performing sentinel node biopsy alone should be credentialed to do sentinel lymph node biopsy alone as well. It seems reasonable to apply the same principle to practicing surgeons. Surgeons learning the procedure can scrub with a mentoring surgeon and a mentoring surgeon can offer his or her expertise to the learning surgeon on the learning surgeon's own patients. (5) Data should be collected and documented and include the surgeons present at the procedure, whether a credentialed surgeon was present, how many sentinel nodes were removed, the characteristics of the sentinel nodes (hot and blue, blue only), the axillary basin count (very important), total number of non-sentinel nodes removed, total sentinel nodes with disease, ~~as well as, the~~ total non-sentinel nodes removed, ~~total sentinel nodes with disease~~, as well as, the total non-sentinel nodes with disease. (6) Once 20 to 30 cases have been achieved the assigned and experienced surgeon reviews the results; ~~paying close attention to~~ the false-negative sentinel node found in the setting of bulky axillary disease may be disregarded as resulting from anatomic problems not from the surgeon's inexperience with the technique.

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APPENDIX

Revised Consensus Statement on Guidelines for Performance of Sentinel Lymphadenectomy for Breast Cancer

Sentinel lymphadenectomy (SL) is a minimally invasive staging procedure for patients with breast cancer. SL, when performed in conjunction with axillary dissection, enhances staging accuracy by identifying occult deposits of microscopic disease that are undetected by routine histological examination of the standard axillary dissection specimen. SL, when performed in lieu of axillary dissection, is associated with less morbidity, and is potentially more cost effective. Multiple studies from tertiary centers with a high volume of experience, and the data from two national registries of community surgical experience show that SL when performed by experienced surgeons, is of equivalent or superior diagnostic staging accuracy. However, the degree of experience required to reliably and accurately perform the procedure, while becoming better understood, is not completely defined.

In regard to SL for breast cancer, the American Society of Breast Surgeons has updated and revised our prior statement. As of September 1, 2000, the American Society of Breast Surgeons acknowledges the following:

1. Patients with palpable, suspicious, metastatic axillary

lymph nodes should not be considered for SL. In addition, SL may be unreliable for patients with multifocal malignancies, for those patients with a history of previous chemotherapy or radiation therapy for breast cancer, and for patients with histories of either extensive prior breast or axillary surgery. SL in this setting should be performed only as part of a research protocol.

2. Axillary treatment for patients with metastatic disease found in sentinel lymph nodes remains controversial. Until further multicenter trial results are available a staging level I and II lymph node dissection is recommended outside of the clinical trial setting.

3. The credentialing and privileging of SL, as with any surgical procedure, are by the policies and processes of each local hospital. Each hospital will define its own criteria for accepting the findings of SL in lieu of axillary dissection and it is encouraged this is done in partnership with an experienced staff breast surgeon. The Society recognizes the controversy regarding the level of experience sufficient for accepting the results of SL as the staging procedure of choice in the clinical setting where the results are used to determine indications for systemic therapy. Information from two national registries quantifying the community experience was presented at the year 2000 annual meeting of the American Society of Breast Surgeons. Findings from these registries indicated that an approximate 10 case experience is necessary for a >85% success in identifying an axillary sentinel lymph node. More importantly, data from these two data bases indicate that an individual surgical experience of at least 20 cases of SL, where both SL and axillary dissection are performed, is necessary to minimize the risk of false-negative results. The false-negative rate (ie, the ratio of the number of false-negative biopsies to the number of patients with positive lymph nodes) is most important factor regarding accurate sentinel lymph node staging. Past experience suggests an acceptable average false-negative rate in the range of 5%.

4. The impact on a surgeon's experience by proctored cases, and formal training in continuing medical education accredited courses is thought to reduce the personal case experience necessary to achieve optimal results, but is yet to be quantitated.

5. After abandoning axillary dissection in favor of SL, surgeons should continue to report their axillary recurrence rate. This rate should be less than 5%. Surgeons are encouraged to report their experience by contributing to national registries and enrolling patients in clinical trials.

Consensus Panel Members:
Michael Edwards, MD
Armando Giuliano, MD
Douglas Reintgen, MD
Lorraine Tafra, MD

SCIENTIFIC PAPERS

000 **Credentialing Issues with Sentinel Lymph Node Staging for Breast Cancer**

Lorraine Tafra, Kelly M. McMasters, Patrick Whitworth, and Michael J. Edwards

Sentinel lymph node staging for breast cancer is accurate, less invasive, and cost effective in the hands of experienced surgeons. The development of credentialing criteria is, however, a subject of significant controversy and opinion. The most important consideration for the acceptance of sentinel lymphadenectomy in lieu of axillary dissection is the false-negative rate.

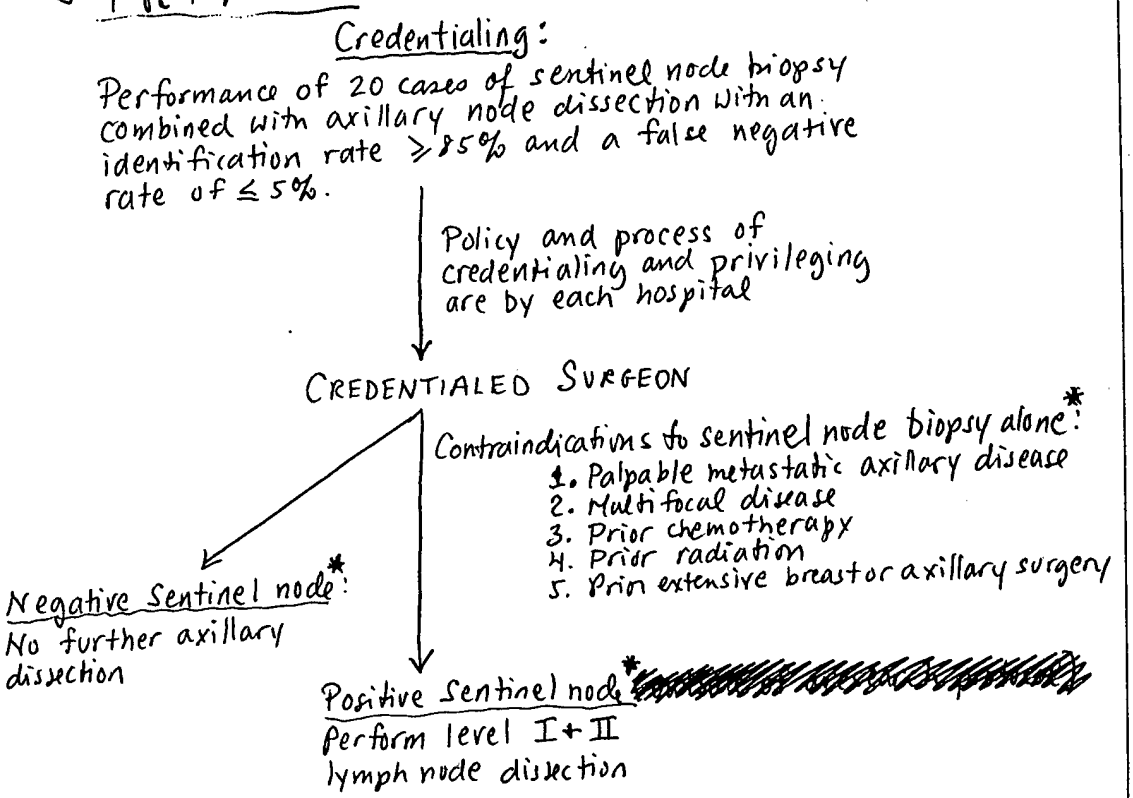
AQ—1 AUTHOR: Please provide Figure 2, "Credentialing Guidelines." Should it be another Appendix, rather than Figure?

AQ—2 AUTHOR: Any volume number? No

AQ—3 AUTHOR: Name of journal? Arch. Surg.

AQ—4 AUTHOR: Can you update now? I have been told by the publisher it will be published in January of 2001

For Figure 2



* Outside of a research protocol

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STATE OF AFFAIRS OF SENTINEL NODE BIOPSY FOR BREAST CANCER

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SENTINEL NODE BIOPSY AND BREAST CANCER: A MULTICENTER VALIDATION STUDY

David Krag, M.D.,Donald Weaver, M.D.,Takamaru Ashikaga, PH.D., Frederick Moffat, M.D., V.Suzanne Klimberg, M.D., Craig Shriver, M.D., Sheldon Feldman, M.D., Roberto Kusminsky, M.D., Michele Gadd, M.D., Joseph Kuhn, M.D., Seth Harlow, M.D., and Peter Beitsch, M.D., *New England Journal of Medicine* 1998; Volume 339, p. 941 – 946.

Objective: To validate the sentinel node concept in breast cancer that the hot node removed from the axilla is the node most likely to contain metastatic disease in breast cancer patients.

Design: Prospective, non-randomized multicenter clinical trial.

Setting: Academic and private practice centers.

Participants: Participating surgeons were required to receive training and perform five training procedures. Participating patients included those with invasive breast cancer, clinically negative axillary lymph nodes and a treatment plan that included axillary lymph node dissection. Exclusion criteria included the presence of clinically suspicious axillary lymph nodes, pregnancy, previous axillary lymphadenectomy or multiple primary tumors.

Results: A total of 443 patients were enrolled in the study. Only technetium-99m sulfur colloid was used for the peri-tumor injection for sentinel node identification. The overall rate of finding a hot spot was 93% or 413/443 patients (the actual identification rate for a sentinel node was 91%). The false negative rate was 11.4% or 13 patients with a false negative sentinel node with a total of 114 patients that had disease in their axilla. The individual investigator identification rate of finding a hot spot prior to the incision ranged from 79% to 98%. The false negative rate ranged from a low of 0% to as high as 29%. This did not statistically vary among the surgeons due to the few numbers of false negative

results actually obtained. A univariate analysis of the false negatives found that only location of tumor in the lateral half of the breast appeared to increase the number of false negative results. The overall rate of identification did vary significantly according to the surgeon. There was, however, no significant difference on univariate analysis of the size, location, histological type, method of injecting the tracer, interval between injection and surgery, or whether the patient had a prior biopsy. A multivariate analysis, however, did reveal that age greater than fifty years, as well as, undergoing a previous excisional biopsy and the primary tumor in the medial location, were associated with failure to find the sentinel node.

Authors' Conclusions: Sentinel node biopsy can predict the presence or absence of axillary node metastases in patients with breast cancer, however, there are characteristics of both the tumor as well as the surgeon that can influence the success of the procedure.

Reviewer's Comments: This is a landmark article, which reviews the first multicenter results of sentinel node biopsy for breast cancer. It validated the concept introduced by Giuliano and Morton [1,2] that the hot and or blue node draining a tumor is the first node to receive metastatic disease and therefore the node most likely to contain disease. As all the other prior studies were single institution series, this also validated that the technique could be performed at multiple institutions and in multiple clinic settings. It also brought to light that although the initial published single institution series had a very low false negative rate, the rate could vary tremendously depending on the surgeon, in this trial, reaching as high as 29%. Patients over the age of fifty, according to this study, will have a lower identification rate thereby eliminating a large group of breast cancer patients from potentially benefiting from sentinel node biopsy alone if a solution is not found. In addition, a significantly lower identification rate was also found in patients who had undergone a prior open biopsy.

SENTINEL LYMPH NODE BIOPSY AND AXILLARY DISSECTION IN BREAST CANCER: RESULTS IN A LARGE SERIES

Umberto Veronesi, Giovanni Paganelli, Giuseppe Viale, Viviana Galimberti, Alberto Luini, Stefano Zurrida, Chris Robertson, Virgilio Sacchini, Paolo Veronesi, Enrico Orvieto, Concetta De Cicco, Mattia Intra, Giampiero Tosi, Daniela Scarpa. *Journal of the National Cancer Institute* 1999; Volume 91, p. 368 –373.

Objective: To validate and clarify the role of sentinel node biopsy to predict the presence of axillary metastases in the management of breast cancer.

Design: Prospective, non-randomized clinical trial.

Setting: Single institution including patients treated at the European Institute of Oncology.

Participants: Patients undergoing complete axillary node dissection excluded for pregnancy, lactation, non-infiltrating carcinoma and previous excisional biopsy. Patients were also excluded if metastatic involvement of the axilla was present or suspected.

Results: The method of sentinel node biopsy included technetium-99m sulfur colloid particles of human albumin injected subdermally if the tissue was superficial and peri-tumorally if the tissue was deep. Sentinel node biopsy was performed 14 – 20 hours following the injection. The identification rate was 99% with a sentinel node identified in 371 of 376 patients enrolled. There were 12 false negative results for a false negative rate of 6.7%. Thirty percent of the patients had lesions greater than 2 cm. A fisher's exact test looked at variables associated with the false negative rate and neither multicentricity, age, tumor size, proliferative index, or estrogen and progesterone receptor status was associated with increased false negative rate. Data was also presented on the total absorbed dose of radioactivity for the clinical staff in a hundred consecutive breast cancer procedures. All effective doses to the surgeon and pathologist with respect to hands and

lens of eye were below annual dose limits for the general population recommended to be safe.

Author's Conclusion: Sentinel node biopsy, using a gamma probe, allows staging of the axilla with high accuracy in patients with breast cancer.

Reviewer's Comments: This group of investigators was one of the first to report using a skin injection to determine the sight of the sentinel node for breast disease. Their initial series reported a significantly higher identification rate than that previously reported in other singles institution series. In addition, their false negative rate remained quite low. These results differ significantly from the previously reviewed article which showed a decreased identification with older age (>50), as this factor had no influence on success rate in this series.

LESSONS LEARNED FROM 500 CASES OF LYMPHATIC MAPPING FOR BREAST CANCER

Arnold D.K. Hill, MCh.,FRCSI, Katherine N. Tran,BA, Tim Akhurst, MD, Henry Yeung, MD, Samuel D.J. Yeh, MD, Paul Peter Rosen, MD, Patrick I. Borgen, MD, and Hiram S. Cody III, MD. *Annals of Surgery* 1999; Volume 229, p. 528 – 535.

Objective: To evaluate the factors affecting the identification and accuracy of sentinel node in breast cancer patients in a single institution series.

Design: Prospective, non-randomized single institution academic center.

Participants: Patients with invasive breast cancer treated with mastectomy and breast conserving surgery. Patients were excluded if they were pregnant or clinically node positive. Any size lesion from T1- 3 was included.

Results: Sentinel node biopsy was performed by using unfiltered technetium-99m sulfur colloid injected peri-tumorally in the breast parenchyma in the majority of cases, however, a select group of patients were also treated intradermally and parenchymally with the isotope injection. At the time of surgery isosulfan blue was also injected and the standard lymph node dissection was only performed in the first sixty patients. The identification rate for the series was 92%, 458 of 492 and the mean number of sentinel nodes removed was 2.1. The overall false negative rate was 10.6% or 5/47 patients with positive lymph nodes of the patients that underwent conventional axillary node dissection.

Author's Conclusions: Sentinel node biopsy in patients with early breast cancer is a safe and effective alternative to routine axillary node dissection for patients with negative nodes.

Reviewer's Comments: This study showed a high identification rate when both isosulfan blue and Tc-99 were used. The study showed a high false negative rate with experienced surgeons. Advancement to performing sentinel node biopsy proceeded despite this false negative rate. Although the results are helpful in examining the identification rate, data on the false negative rate, probably the most important aspect of the technique, could not be obtained from their small group of 49 patients with positive nodes, although they did attempt to look at this data. Interesting data was also presented in this series showing the number of patients that had blue only sentinel nodes, hot only sentinel nodes and blue and hot sentinel nodes in their axilla. There was a relatively equal distribution in these three groups whether a single sight of sentinel nodes, two sights, or three or more sights of sentinel nodes were found.

This data is somewhat confused by the fact that all the patients did not undergo this same technique of injection i.e. approximately 20% of the patients were injected intradermally with the isotope. The overall conclusion appears reasonable that sentinel node biopsy in patients with early breast cancer is safe, however, their conclusion that the overall false

negative rate remains low in the majority of patients, is probably erroneous as they have not looked at the false negative rate in a large group of patients.

Summary:

Numerous single institution series documenting the ability of the sentinel node to predict the presence of disease in the remaining lymph node basin were published prior to Krag et al., the first article presented in this series. Its significance lies in its method and vision in realizing the need to validate the concept behind the sentinel node technique in a multicenter trial. The second paper was chosen, as it established a different technique (injection of the skin) as an accurate method to achieve adequate, accurate identification of the sentinel node. As the identification was higher than other single institution series that came before it, the question was appropriately raised if the technique was in fact superior to the peri-tumoral injection. There has been no randomized trial to evaluate the two techniques. The third article attempts to determine those factors associated with the identification rate of the sentinel node.

Overall, the impact of sentinel node biopsy on the management of breast cancer has been tremendous. Sixty percent of breast cancer patients do not harbor metastatic disease in their lymph nodes. Potentially being able to eliminate a morbid procedure such as axillary node dissection and replace it with a simple axillary node biopsy is having an impact on patients that is equivalent to replacement of mastectomy with breast sparing surgery.

Although the technique of sentinel node biopsy began with melanoma, there are clear differences in performing the technique for breast cancer. The false negative rate for the initial series of breast cancer patients was significantly higher than the initial series for

melanoma. The two initial academic and private practice single institution series presented by Guiliano et al and Guenther et al showed a false negative rate of 12 and 10% respectively [1,3]. This is significantly higher than the original series for melanoma which was reported between 3% and 5% from the Sydney Melanoma Unit and the John Wayne Cancer Institute [2,4]. The technique for breast cancer has a significantly more difficult learning curve than when performed for melanoma.

What is the acceptable level of false negative rate for breast disease? The clinical implication of missed metastatic disease for breast cancer is possibly greater than that for melanoma. The reasons for this include a lower rate, in general, of developing metastatic disease (20% versus 40%) and the increased difficulty of finding the correct node in breast cancer. The data that continues to be scarce are those factors that significantly influence the false negative rate. The only studies that can evaluate these factors are those studies in which patients undergo a complete axillary lymph node dissection. Only the two trials reviewed in this set of three can provide insight into factors that may affect the false negative rate and even these series are too small to definitively predict which factors are important.

There are a number of issues that remain to be resolved with this new surgical technique. Questions that remain include 1) who is a candidate for sentinel node biopsy and what factors influence the accuracy of the procedure? 2) What are the contraindications of sentinel node biopsy? 3) What is the best technique? 4) How many procedures need to be performed by any one surgeon prior to abandoning complete axillary node dissection for sentinel node biopsy alone? 5) Is there any role for sentinel node biopsy for DCIS? 6) Should internal mammary sentinel nodes be dissected? 7) How should the sentinel node be analyzed by pathology? 8) Should all patients with a positive sentinel node undergo complete lymph node dissection?

Who is a candidate for sentinel node biopsy or what factors influence the accuracy of the procedure?

The factors that are worth investigating include the age of the patient, the patient's prior surgery (open biopsy versus lumpectomy), the technetium-99m sulfur colloid preparation used, the injection time intervals of technetium-99m sulfur colloid, tumor size, tumor location, surgeon's level of experience, probe type and amount of disease in the axilla.

Age as an important factor

Age has been found by Krag et al. to be an important factor in the identification rate of finding the sentinel node. The older the patient the lower the identification rate. It can be hypothesized that as the patient ages the breast is steadily replaced by fat and the lymphatic density decreases. It may also be that sluggish lymphatics delay the concentration of the technetium-99m sulfur colloid in the sentinel node and make it difficult to find. With the introduction of the intradermal injection, and its probably higher identification rate, this factor may become less important. Older patients should be informed that they might have a higher non-identification rate and a greater chance of requiring a complete lymph node dissection (if it is not found).

Tumor Size as a factor

The majority of series have performed sentinel node biopsy on tumor sizes ranging from T1-3 with success. The number of patients with the larger size tumors remains relatively small and definitive conclusions can only be drawn with larger studies. Two studies have specifically looked at larger tumors and these showed no difference in either the

identification or the false negative rate in a group of patients undergoing complete lymph node dissection [5,6].

Prior surgery of the breast as a factor

There are a number of studies that have documented no difference in the results of sentinel node biopsy for breast cancer in patients that have a prior breast biopsy [7,8] however Krag et al. and others (all of which have been small series) have found either a higher false negative rate or lower identification rate [9]. Tumors that remain intact and or diagnosed by core biopsy that are nonpalpabe have an excellent success rate [10]. As there has been a general trend to diagnosing tumors more frequently with core biopsy devices this may become less of an issue.

What are the contraindications of sentinel node biopsy?

It has been stated consistently that definite contraindications for sentinel node biopsy include patients with clinically positive axillary nodes. These patients benefit from completion axillary node dissection and *just* a sentinel node biopsy would be inadequate treatment. Controversial contraindications include patients that have received preoperative chemotherapy or radiation therapy. It needs to be determined in a large group that these factors do not negatively influence the results of sentinel node biopsy.

What is the best technique?

Where to inject:

The Milan study showing a high identification rate with the intradermal injection has now been confirmed in a number of other studies [11]. Sappey's plexus or the subareolar plexus has also been reported with excellent results [12,13]. This is felt to be superior to a peri-timor injection: in patients with an upper outer quadrant lesion there can be significant shine through from the injection of the radiocolloid making identification of a sentinel node difficult. Is there discordance or different drainage patterns when a tumor is injected either in the peri-tumorally area, the skin or Sappey's plexus? Larger studies are needed to answer the question. One group has injected all three sites (Sappey's plexus, skin, and peritumor) with three agents to attempt to answer the question. Their results in a small number of patients show that all injection sites drain most of the time to the same sentinel nodes – but not always (personal communication, Dr. Donald Lannin, East Carolina University).

Dyes Used:

The most commonly used dye is isosulfan blue, however, other agents including fluorescein, indigo carmine [14, 15] and indocyanide green [16] have been used.

Timing and preparation of Technetium-99m sulfur colloid used and whether or not to image:

A number of different radiocolloids have been used and all with good results. One study injected a patient twice and the agent that successfully identified a sentinel node had a larger particle size [17]. Another study used a dose ten times what is typically used in the United States and reported no increase in shine through, an excellent identification rate, and a very high rate of internal mammary sentinel node identification.

The optimal timing of Technetium-99m sulfur colloid has not been well documented but logically should allow enough time for the technetium-99m sulfur colloid to concentrate in the sentinel node but not enough time for it to pass through to the other nodes or dissipate. A wide variety of time intervals have been used from very short to overnight [18-20] with good success. Some studies have found no significant differences with varying timing intervals [21]. All patients with melanoma are imaged prior to sentinel node biopsy, as the incidence of discordance of expected drainage sites remains high. Although most investigators began imaging in preparation for using sentinel node biopsy for breast cancer, it was found to be less successful than for melanoma. Its use with medial quadrant lesions to identify internal mammary node drainage remains to be determined. Some studies have found that it serves no purpose [22].

How many procedures need to be performed by any one surgeon prior to abandoning complete axillary node dissection for sentinel node biopsy alone?

A consistently observed finding in most studies is a learning curve which shows improved identification of the sentinel node as well as decreases in false negative rates as surgeons increase their experience [23]. A hypothetical statistical analysis has suggested that in order for the procedure to be cost effective an identification rate over 80% needed to be documented [24]. The American Society of Breast Surgeons released a consensus statement stating that an experience with at least 30 patients completing a full node dissection with a false negative rate less than 5% was acceptable to then proceed to performing sentinel node biopsy alone [25]. It raised a number of issues therefore as to the level of expertise that must be obtained prior to performing sentinel node biopsy alone without a completion axillary node dissection.

Is there any role for sentinel node biopsy for DCIS?

Performing sentinel node biopsy for DCIS remains controversial but more data is surfacing showing a small percentage of patients will have metastatic disease [26,27]. The clinical significance of this finding remains unclear in this population of patients who rarely die of their breast cancer. More frequently patients are being diagnosed with DCIS by stereotactic core biopsy thereby obtaining their diagnosis through a sampling of a mammographic abnormality. How often this sampling gives the entire picture of the lesion has important implications in the use of sentinel node biopsy for these patients. If the stereotactically diagnosed DCIS patient elects mastectomy and subsequently it is discovered that she has an invasive lesion, she has lost the opportunity for sentinel node biopsy. The challenge is determining those DCIS patients that have a high enough chance of harboring invasive disease to make performance of sentinel node biopsy worthwhile.

Should internal mammary sentinel nodes be dissected?

Few studies have reported extensive experience dissecting sentinel nodes from the internal mammary chain [28]. It is tempting to speculate that our level of surgical sophistication has increased enough to allow dissection of only clinically significant lymph nodes in this elusive site with little morbidity.

How should the sentinel node be analyzed by pathology?

There is no question that the ability to give the pathologist one or two nodes upon which to direct all his/her attention increases the sensitivity of finding metastatic disease and this has been well documented in the literature [29-35]. It remains to be determined if this increased sensitivity translates in improved accuracy in prognosis. There are a significant number of patients that have historically been node negative that have died of their cancer.

There is little doubt that there is room for improvement in diagnostic accuracy. Whether routine immunohistochemistry should be used on sentinel nodes remains controversial as does investigational use of polymerase chain reaction detection [36-42].

Should all patients with a positive sentinel node undergo complete lymph node dissection?

Standard of care would dictate that a full dissection is necessary if a sentinel node contains metastatic disease. If a patient has only a microscopic deposit or an immunohistochemistry positive deposit the standard of care has not been determined. In one study only 7% of patients had addition non-sentinel nodes positive when the patient had a micrometastases in the sentinel node [29]. Further studies will need to better establish the incidence on non-sentinel node metastases with micrometastases in the sentinel node.

What if a sentinel node cannot be found? Guenther et al [43] has made an interesting observation that patients with an unidentifiable sentinel node have a 34% rate of having positive sentinel nodes. Until further data is obtained it stands to reason that when a sentinel node cannot be identified a completion axillary dissection should be performed.

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Revised Consensus Statement on Guidelines for Performance of Sentinel Lymphadenectomy for Breast Cancer

Original statement released November 1998.

Sentinel lymphadenectomy (SL) is a minimally invasive staging procedure for patients with breast cancer. SL, when performed in conjunction with axillary dissection, enhances staging accuracy by identifying occult deposits of microscopic disease that are undetected by routine histological examination of the standard axillary dissection specimen. SL, when performed in lieu of axillary dissection, is associated with less morbidity and is potentially more cost effective. Multiple studies from tertiary centers with a high volume of experience, and the data from two national registries of community surgical experience show that SL, when performed by experienced surgeons, is of equivalent or superior diagnostic staging accuracy. However, the degree of experience required to reliably and accurately perform the procedure, while becoming better understood, is not completely defined.

In regard to SL for breast cancer, the American Society of Breast Surgeons has updated and revised our prior statement. As of September 1, 2000 the American Society of Breast Surgeons acknowledges the following:

1. Patients with palpable, suspicious, metastatic axillary lymph nodes should not be considered for SL. In addition, SL may be unreliable for patients with multifocal malignancies, for those patients with a history of previous chemotherapy or radiation therapy for breast cancer, and for patients with histories of either extensive prior breast or axillary surgery. SL in this setting should be performed only as part of a research protocol.

2. Axillary treatment for patients with metastatic disease found in sentinel lymph nodes remains controversial. Until further multi-center trial results are available a staging Level I and II lymph node dissection is recommended outside of the clinical trial setting.

3. The credentialing and privileging of SL, as with any surgical procedure, are by the policies and processes of each local hospital. Each hospital will define its own criteria for accepting the findings of SL in lieu of axillary dissection and it is encouraged that this is done in partnership with an experienced staff breast surgeon. The Society recognizes the controversy regarding the level of experience sufficient for accepting the results of SL as the staging procedure of choice in the clinical setting where the results are used to determine indications for systemic therapy. Information from two national registries quantifying the community experience was presented at the year 2000 annual meeting of the American Society of Breast Surgeons. Findings from these registries indicated that an approximate 10 case experience is necessary for a ≥85% success in identifying an axillary sentinel lymph node. More importantly, data from these two databases indicates that an individual surgical experience of at least 20 cases of SL, where both SL and axillary dissection are performed, is necessary to minimize the risk of false-negative results. The false-negative rate (i.e., the ratio of the number of false-negative biopsies to the number of patients with positive lymph nodes) is the most important factor regarding accurate sentinel lymph node staging. Past experience suggests an acceptable average false-negative rate in the range of 5%.

4. The impact on a surgeon's experience by proctored cases, and formal training in accredited continuing medical education courses is thought to reduce the personal case experience necessary to achieve optimal results, but is yet to be quantitated.

5. After abandoning axillary dissection in favor of SL, surgeons should continue to report their axillary recurrence rate. This rate should be less than 5%. Surgeons are encouraged to report their experience by contributing to national registries and enrolling patients in clinical trials.

Approved August 25, 2000

The American Society of Breast Surgeons

Consensus Statement on Guidelines for Performance of Sentinel Lymph Node Biopsy for Breast Cancer

Sentinel lymph node biopsy (SLNB) is a minimally invasive procedure for staging patients with breast cancer. SLNB, when performed in conjunction with axillary dissection, enhances staging accuracy by identifying occult deposits of microscopic disease that are undetected by routine histological examination of the standard axillary dissection specimen. SLNB, when performed in lieu of axillary dissection, is associated with less morbidity, and is potentially more cost effective. When performed by experienced surgeons, SLNB is highly accurate and reliably reflects the histology of the nodal basin. However, the degree of experience required to reliably and accurately perform the procedure is less well defined.

The most important factor regarding accurate sentinel lymph node staging is the false-negative rate (i.e., the ratio of the number of false-negative biopsies to the number of patients with positive lymph nodes). Single institutions with a high-volume experience report an average false-negative rate in the range of 0–5%. The first reported multi-institutional experience (N Engl J Med 339:941-946, 1998) documents a slightly greater than 11% false-negative rate, with individual surgeons as high as 28%.

In regard to SLNB for breast cancer, the American Society of Breast Surgeons recommends the following:

Patients with palpable, suspicious, metastatic axillary lymph nodes are ineligible for SLNB. In addition, patients with multifocal malignancies, tumors >5 cm, or a history of previous chemotherapy or radiation therapy for breast cancer are also ineligible. Patients with histories of either extensive prior breast or axillary surgery may likewise have altered lymphatic drainage, limiting the accuracy of SLNB.

To accept the findings of sentinel lymph node biopsy alone in lieu of axillary dissection, each surgeon, with his or her collaborating institution (pathology, radiology, and nuclear medicine colleagues, etc.) should document an individual experience as surgeon or first assistant of ≥ 30 cases where both SLNB and axillary dissection are performed. This experience should yield (1) a $\geq 85\%$ success rate in identifying an axillary sentinel lymph node, and (2) a false-negative rate $\leq 5\%$, or a single false-negative sentinel lymph node in the series.

This level of accuracy is, in the opinion of the society, sufficient for accepting the results of SLNB as the staging procedure of choice in the clinical setting where the results are used to determine indications for systemic therapy.

Axillary treatment for patients with metastatic sentinel lymph nodes remains controversial. Until further multicenter trial results are available, complete lymph node dissection is recommended.

After abandoning axillary dissection in favor of SLNB, surgeons should continue to report their axillary recurrence rate. This rate should be less than 5%. Surgeons are encouraged to report their experience by contributing to national registries and enrolling patients in clinical trials.

Consensus Panel Members
Michael Edwards, MD
Armando Giuliano, MD
Douglas Reintgen, MD
Lorraine Tafra, MD

125 RT-PCR INCREASES DETECTION OF BREAST CANCER SENTINEL LYMPH NODE (SLN) MICROMETASTASES. Verbanac KM*, Fleming TP, Min CJ, Purser SM, Tafra L. East Carolina University, Greenville, NC 27858 *and* Washington University, St. Louis, MO 63110.

RT-PCR analysis of SLN offers the promise of improved staging sensitivity over routine histological methods, which fail to detect disease in many patients. We have previously identified mammaglobin and CEA as superior molecular markers for RT-PCR detection of occult breast disease (*Cancer Res* 58:4581,1998; *Proc Soc Surg Oncol* #P16,1999). Here we report RT-PCR analysis of an initial cohort of patients enrolled in our multicenter trial, comparing 3 levels of detection sensitivity.

We analyzed 123 LN from 49 patients for mammaglobin mRNA. Alternate serial sections of each LN were designated for histology (histo) (H&E ± immunohistochemistry) or RT-PCR. PCR criteria included ≥2 PCR reactions from ≥2 cDNA preparations and stringent controls. PCR products were identified by ethidium bromide (EtBr) stain. To increase detection sensitivity, selected specimens were re-examined using 2X cDNA template & EtBr and/or by Southern blot (SoBt) analysis.

31/34 histo+ LN were PCR+ with 1X cDNA & EtBr. Two remaining LN were PCR+ with 2X cDNA & EtBr (3% false negatives by LN; 23/23 histo+ patients were PCR+). 13/89 histo– LN were PCR+ with 1X cDNA & EtBr, upstaging 4/26 patients. An additional 16 LN (9 histo– patients) were + with 2X cDNA & EtBr. Thus, mammaglobin RT-PCR analysis may upstage as many as 12/26 histo– patients (46%). This is consistent with the recurrence rate generally observed in histo– patients. SoBt identified an additional 3 LN as PCR+, but no patients were upstaged. No normal LN were mammaglobin PCR positive at any sensitivity level.

Mammaglobin PCR is a specific, sensitive method to detect breast SLN micrometastases. Our ongoing analysis for CEA may further increase detection of occult disease. This study points out the importance of evaluating different molecular detection sensitivities in prospective, blinded clinical trials. Long term follow-up for recurrence will be required to determine which molecular detection level is clinically significant.

126 LOCALISATION OF SENTINEL AXILLARY LYMPH NODES IN BREAST CANCER PATIENTS. OUR EXPERIENCE.
Yong WS*, Wong CY, Lee SY, Ng EH, Soo KC, Tan PH, G Chiang, A Goh. Departments of Surgery , Pathology and Nuclear Medicine, Singapore General Hospital, Singapore 169608.

Axillary dissection is currently the standard of care for treatment of breast cancer to determine prognosis and need for adjuvant therapy. Sentinel axillary lymph node biopsy has been advocated as a less morbid alternative to determine axillary lymph node status.

Our aim is to determine the feasibility of sentinel lymph node detection using radio-isotope tracking and vital blue dye, and to determine the accuracy of the sentinel lymph node in predicting the status of the axillary lymph nodes.

A total of 312 patients with breast cancer were accrued from August 1996 to December 1998. After the sentinel lymph node was localised and removed, a standard wide excision or mastectomy was performed with axillary dissection.

Sentinel lymph node biopsy was found to have a sensitivity of 83%, specificity of 100% and accuracy of 93% in predicting axillary lymph node status. The false negative rate is 16.7%. The success rate of locating the sentinel lymph node is 86%.

We conclude that sentinel lymph node biopsy is feasible, accurate and can be performed but still carries a high false negative rate and therefore cannot as yet be offered as a standard treatment for breast cancer patients.

127 **COMPETING RISKS ANALYSIS FOR RECURRENCE FROM PRIMARY BREAST CANCER AFTER LUMPECTOMY.** **Fan M[1], Chapman JW[1,2]*, Link MA[2], Fish EB[2]. [1]Department of Public Health Sciences and [2]Henrietta Banting Breast Centre, Women's College Hospital, University of Toronto, Toronto, Ontario, Canada.**

We previously showed that assessment of competing risks of recurrence was important for all breast cancer patients. We have now examined competing risks of recurrence for women who underwent lumpectomy. A cohort of 366 patients who had lumpectomy was accrued between 1971-1990. These patients had unilateral primary invasive breast cancer with no previous history of carcinoma, except possibly in situ cervix or non-melanoma skin. The patients were followed to 1996. Factors assessed are age (in years), tumor size (in cm), nodal status (N-,Nx,N+), ER (fmol/mg protein), PgR (fmol/mg protein), adjuvant radiotherapy (no, yes), adjuvant hormonal therapy (no, yes), adjuvant chemotherapy (no, yes). We examined the risk factor effects with non-parametric, semi-parametric, and parametric (i.e., log-normal) analyses. The shapes of the hazard functions for the three types of recurrence are clearly different, and there are different factor associations by type of recurrence. For Cox and log-normal models, older age was associated with better local (p=0.02, p=0.02) and distant (p<0.001, p=0.002), and worse regional (p=0.07, p=0.06) disease-free survival (DFS). Adjuvant radiotherapy led to longer local DFS (p=0.002, p=0.01), and had no effect on regional recurrence by either model type (p>0.1). Surprizingly, radiotherapy was associated with significantly better distant DFS (p=0.01) with a Cox model. Adjuvant chemotherapy led to better local (p=0.03, p=0.01) and distant (p=0.01, p=0.02) DFS, while having no effect on regional recurrence (p>0.1). Patients with larger tumor size (p<0.001, p<0.001) and lymph node involvement (p=0.007, p=0.03) had shorter DFS. Competing risks analysis improves the assessment of prognostic factor effects.

128 HER3 (c-*erb*B-3) EXPRESSION IN BREAST CANCER PREDICTS SURVIVAL AND IS RELATED TO HER2 EXPRESSION. Reeves JR*[,], Cooke TG and Stanton PD[#]. University Department of Surgery, Royal Infirmary, Glasgow, Scotland. Now at: [,]London Regional Cancer Centre, 790 Commissioners Road E, London, Ontario, Canada N6A 4L6 and [#]Discipline of Surgery, University of Tasmania, Box 252-28, Hobart, 7001, Australia.

Previous studies have examined a total of ~1500 primary breast tumours for HER3 expression with little consistency in findings. About 80% of these cases have been assessed with the RTJ1 monoclonal antibody. Concerns have been voiced regarding the specificity of RTJ1, so here we assessed a panel of anti-HER3 antibodies for specificity and applied one to measure HER3 in 211 frozen primary breast cancers previously assessed for HER2 expression.

Frozen pellet sections of HER3 cells (transfected with c-*erb*B-3 cDNA) were labelled immunohistochemically with the RTJ1, RTJ2, SGP1, 90.6 and 105.5 monoclonal antibodies. RTJ1 gave no specific labelling but the other antibodies labelled intensely the HER3 cells but not the untransfected parent line. In frozen breast cancers, 105.5 gave the strongest labelling, and as this antibody recognises the ligand binding domain of HER3, specificity was further confirmed by preincubation of sections with excess heregulin β1 resulting in a reduced labelling, whereas excess epidermal growth factor did not.

211 frozen breast cancers were labelled immunohistochemically with the 105.5 antibody. Scoring was on a 4 point scale (0 to 3 +). Labelling was cytoplasmic with membrane accentuation with 15% of cases negative, 32% 1+, 36% 2+ and 18% 3+. Univariate survival analysis indicated that both the negative and 3+ categories had poor overall (p = 0.015) and disease free survival (p = 0.0001) when compared to the 1+ and 2+ groups. HER3 expression was positively correlated with the expression of HER2 (p < 0.0005).

This study suggests that HER3 may indeed have a role in the biology of breast cancer and co-expression with HER2 may indicate that these two molecules work in combination rather than in isolation. Further work is required to assess whether HER3 expression analysis can add to the prognostic value or the predictive value of HER2 in determining the response to targeted therapy

9 AN EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) TYROSINE KINASE INHIBITOR (ZD1839) INHIBITS PROLIFERATION IN NORMAL AND PREINVASIVE EPITHELIA. Chan KC*, Knox WF, Woodburn JR[1], Potten CS, Bundred NJ. University Hospital of South Manchester, and Zeneca Pharmaceuticals[1], UK.

Estrogen receptor (ER) negative ductal carcinoma in situ (DCIS) expresses the cerbβ_2 and epidermal growth factor receptors. We sought to determine if blocking of EGFR with an EGFR tyrosine kinase inhibitor (ZD1839) prevents heterodimerization of EGFR with the cerbβ_2 receptor and signal transduction of both receptors leading to decreased epithelial proliferation in normal and DCIS human breast tissue.

Normal and DCIS tissue from 8 women undergoing surgery were implanted into 16-20 athymic nude mice per experiment (8 xenografts/mouse). Daily gavage with either ZD 1839 at 100-200mg/Kg or vehicle for 14 days commenced 2 weeks post implant. Xenografts were removed on days 14, 21, and 28. Epithelial proliferation was assessed by counting 1000 cells after Ki67 immunostaining. ZD1839 increased apoptosis ($p<0.05$) after 7 days of treatment and inhibited proliferation compared to controls after 14 days ($p<0.05$; see table).

Ki67	Day 0	Day 21		Day 28	
Labelling Index (%)		Control	ZD1839	Control	ZD1839
DCIS Median	19.9%	10.6%	7.2%	23%	3.2%*
Interquartile range	5.7-30.9	2.2-39.2	0.5-21.2	2.2-39.2	0-8.3
Normal breast					
Median	7.6%	3.3%	0.7%*	6.1%	1.8%*
Interquartile range	4.4-12.9	0.8-4.8	0-1.3	3.4-7.8	0.7-3.9

*p<0.05

The growth of MDA MB 231 breast cancer cells was also inhibited *in-vitro* at 1μM of ZD1839 and *in-vivo* at 200 mg/kg ($p<0.05$).

ZD1839, an EGFR tyrosine kinase inhibitor, has potential as a chemopreventative agent and as adjuvant therapy in ER negative DCIS.

10 IMPACT OF MICROMETASTASES ON THE SURVIVAL OF WOMEN WITH T1 BREAST CANCER. DC Maibenco*[1], LK Weiss[2], JJ White[1], TY Kau[2], RK Severson[2]. Surgical Specialists of Decatur, Decatur, IL[1]. Karmanos Cancer Institute, Detroit, MI[2].

The most important factor in predicting survival among women with breast cancer is the status of the axillary lymph nodes. The impact of micrometastases on survival is contentious. This study was performed to determine the impact of micrometastases on the survival of women with T1 breast cancer.

A review of data from the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute was performed using cases diagnosed from January 1988 through December 1995. Among women with invasive breast carcinomas ≤2 cm undergoing a resection of the primary malignancy and an axillary lymph node dissection there were 30,168 cases without lymph node metastases, and 744 cases with micrometastases, pathologic N1a lymph node metastases. Survival differences among these two groups were calculated using actuarial survival rates.

Among women with T1 breast cancer and micrometastases decreased survival was associated with increasing histologic grade, and older age. Survival was adversely effected by the presence of an increasing number of lymph nodes with micrometastases ($p=0.032$). Micrometastases were associated with a survival disadvantage among cases with size ≤1.0 cm ($p=0.0009$), histologic grade 3 & 4 carcinomas ($p=0.002$), similar histology (IDC $p=0.049$; ILC $p=0.12$; Other $p=0.003$), and similar age group (<50 years $p=0.003$; ≥50 years $p=0.009$). Micrometastases were not associated with a survival disadvantage among cases with size 1.1-2.0 cm ($p=0.26$), and histologic grade 1 ($p=0.23$) and grade 2 ($p=0.76$) carcinomas.

Among women with T1 breast cancer and micrometastases five-year survival was lower than in cases without associated lymph node micrometastases in most, but not all subgroups.

11 **CYTOKERATIN-POSITIVE BONE MARROW MICROMETASTASES IN STAGE I-III BREAST CANCER PATIENTS INDICATE POOR PROGNOSIS INDEPENDENTLY OF LYMPH NODE METASTASIS.** Braun S*, Pantel K, Janni W, Hepp F, Kentenich CRM, Müller P, Gastroph S, Dimpfl T, Riethmüller G, and Schlimok G. I. Frauenklinik and Institute of Immunology, Ludwig-Maximilians University, Munich; Frauenklinik, Eppendorf University, Hamburg; II. Med. Klinik, Klinikum Augsburg; Germany.

So far, various markers have been used to diagnose epithelial bone marrow (BM) micrometastases. Of these, cytokeratins (CK) have been shown to be reliable in terms of specificity and sensitivity, which is in clear contrast to largely unspecific epithelial markers, such as members of the polymorphic epithelial mucin family. Since only limited information has been thus far available on the clinical relevance of CK+ breast cancer cells in BM, we investigated whether the detection of such cells is correlated with poor recurrence and poor prognosis.

According to a prospective design, BM aspirates were obtained from 506 patients with stage I-III breast cancer prior to complete tumor resection (stage R0), and stained with the monoclonal antibody A45-B/B3 directed against a common epitope of CKs. CK+ cells in BM aspirates were detected in 183 (36%) of 506 breast cancer patients. The presence of tumor cells was correlated with the diagnosis of inflammatory breast cancer ($P<0.0001$), poor nuclear grading of the primary tumor ($P=0.039$), and histological involvement of > 9 lymph nodes ($P<0.0001$). No correlation was found with lymph node metastasis ($P=0.21$). After a median follow-up of 30 months, DFS and OS rates in the group with BM micrometastases were 28% and 65%, respectively, whereas in 323 patients without such micrometastases, the respective rates were 75% and 92% ($P<0.0001$; log-rank test). Despite this relatively short observation time, the established risk factor of axillary lymph node metastasis when combined with the detection of BM micrometastases was associated with a significantly decreased survival ($P = 0.028$ for node-negative, and $P = 0.0005$ for node-positive patients, respectively). According to the multivariate analysis, the presence of BM micrometastases only was found to be an independent prognostic factor with a 2.51-fold increased relative risk of cancer-related death ($P=0.0028$). The relative risk for disease recurrence for patients with the CK+ BM findings was 2.58 ($P<0.0001$).

We conclude that immunocytochemical detection of CK+ micrometastases in BM is an independent prognostic risk factor in stage I-III breast cancer.

12 AGE AND SURGEON EXPERIENCE: THE ONLY SIGNIFICANT FACTORS CONTRIBUTING TO SENTINEL NODE MAPPING FAILURE IN BREAST CANCER. Ng PC*, Chua AC, Lannin DP, Van Eyk JJ, Swanson MS, Tafra L. East Carolina University School of Medicine, Greenville, NC 27858.

Sentinel lymph node biopsy (SLNB) attempts to stage regional lymphatic disease while limiting morbidity in breast cancer patients (BCP). If this technique is to potentially replace complete axillary node dissection, the factors contributing to SLNB failure require further multi-center analysis. This study defines those factors associated with unsuccessful SLNB.

An IRB-approved multi-center clinical trial (1995-99) involving 28 surgeons enrolled BCP for SLNB followed by a level I/II axillary node dissection. The technique employed peri-tumor injections of technetium 99 sulfur colloid and isosulfan blue dye. No investigator participated in a learning trial prior to participation. For comparison, a univariate and multivariate analyses examined factors contributing to nonsuccess: surgical experience, probe type, primary tumor location and size, method of diagnosis, age, and vascular or lymphatic invasion.

The study compared 330 successfully mapped patients to 70 failed SLNB (15 false-negative and 55 with no SN identified). The analysis found no statistical difference between the two groups in tumor size, tumor location, type of gamma probe, vascular or lymphatic invasion, or method of diagnosis (prior biopsy, lumpectomy, FNA, or core biopsy). The only significant differences were patient age and prior experience. Whereas mapping failure occurred in only 7.8% of those < 50 years, the failure rate equaled 22.1% in patients ≥ 50 years, (odds ratio = 3.12, 95% CI, 1.53-6.38). Surgeons performing <10 cases had 22.6% failures compared to 13.1% for those performing ≥ 10 cases (odds ratio = 2.0, 95% CI, 1.11-3.48). The overall false negative (FN) rate equaled 13.4%. For surgeons performing <10 cases, the FN rate reached 16% compared to 10% for those with ≥ 10 cases ($p<0.05$).

Patient age and surgeon experience are the primary factors contributing to SLNB failure. We recommend that surgeons not abandon complete axillary node dissection until they obtain adequate experience and that they use caution in applying the technique to older patients.

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COMPARISON OF INTRADERMAL (ID) AND SUBCUTANEOUS (SC) INJECTIONS IN LYMPHATIC MAPPING

Terry Kersey MD, Jason Van Eyk BS, Donald Lannin MD, Lorraine Tafra MD. Department of Surgery, ECU School of Medicine, Greenville, NC.

Introduction: Sentinel node biopsy (SNB) for melanoma, with its ID injection, has a higher success rate than SNB for breast cancer, which is typically performed with a SC, pertitumor injection. No study has investigated differences in transit time of agents used in lymphatic mapping from injection site to the sentinel node, that could account for this clinical observation.

Goal: To compare transit time between ID and SC injections with common agents used in lymphatic mapping.

Methods: Forty injection sites on five domestic pigs were used. These sites included bilateral, cervical, forelimb, hindlimb and flank areas. Agents included technetium sulfur colloid (Tc99, filtered and unfiltered), isosulfan blue dye (IB) and flourescein dye (F). At each site both ID and SC injections were made and the transit time to reach the sentinel node was recorded.

Results: Sentinel nodes were identified draining all sites, and found to be either hot, blue, or fluorescent (using a Wood's lamp for identification). Time differences were calculated per centimeter distance from the draining lymph node basin. The table below summarizes the results (sec/cm ±SEM).

Injection Technique	Tc99 (Filtered)	Tc99 (Unfiltered)	IB	F
Dermal	4.8 ±2.5	2.7 ±0.54	10.5 ±3.6	6.98 ±1.45
Subcutaneous	59.0 ±18.8	249 ±14.7	6.3 ±2.3	8.27 ±2.3
P Value	0.02	.008	0.3	0.4

Conclusions: Tc99 dermal injections were significantly faster compared to a SC injection. The slowest and fastest SC injection agent was unfiltered Tc99 and IB, respectively. Dermal injections provide faster transit of lymphatic agents and may improve the success rate when applied to patients with breast cancer.

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Information required for electronic submission of an abstract for the American Radium Society 82nd Annual Meeting:

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ARS member: no
Wish an application: yes
Willing to submit a poster: prefer not

Topic area of the paper: Breast, Treatment Modalities (Surgery)
Type of study: Prospective, non-randomized, multi-center
Total case in population:553
Number evaluable:550
Median follow-up: 26 months
Range of follow-up: 3 – 42 months
Doses/Schedules: N/A

Accuracy of Sentinel Node Biopsy (SNB) for Large Breast Tumors

L. Tafra, D. Lannin, L. Egan, M. Ramirez, S. Watkins.
The Breast Center at Anne Arundel Medical Center, Annapolis, MD and East Carolina University, Greenville, NC

Background: SNB for breast cancer is more frequently being used as a diagnostic tool to determine those patients who may benefit from axillary node dissection. It has been hypothesized that SNB in patients with larger tumors may be inaccurate, as the large volume of tumor blocks lymphatics and lymphatic dyes are misdirected to a false negative sentinel node.

Objective: To compare the results of SNB performed for larger (4 cm or greater) versus smaller (less than 4 cm) breast tumors.

Methods: A multi-center trial, initiated in 1996, performed SNB on breast cancer patients under IRB approval followed, in the majority of patients, by complete lymph node dissection. Patients with any size tumor were included in the trial excluding those with suspicious lymphadenopathy.

Results: A total of 553 patients enrolled and 550 were evaluable with completed pathology. Ninety-three percent (510) of all the patients underwent complete lymph node dissection and positive lymph nodes were found in 27%. Tumor size ranged from 0.1 cm to 9.9 cm with the majority of patients having tumors smaller than 4 cm (470, 92% vs. 40, 8%). As expected, the group with larger tumors had a higher rate of positive sentinel nodes (43%) compared to the smaller tumors (25%). Overall, the identification rate of the sentinel node was not significantly different between the two groups (87.5% vs. 90%, NS). The false negative rate for the entire group was 13% (19/139) and did not differ significantly between those patients with larger (6%, 1/17) vs. smaller (15%, 18/122) tumors.

Conclusions: Size does not appear to be a limiting factor in SNB and can accurately be performed on patients with large tumors. Although larger tumors have a higher rate of positive sentinel nodes, 60% of patients with tumors greater than 4 cm, and clinically negative axillary nodes, do not have positive lymphatic disease and may be spared complete axillary dissection.

Department of Defense Breast Cancer Research Program, Era of Hope 2000, Proceedings, Volume 1

MULTI-CENTER TRIAL OF RT-PCR DETECTION OF SENTINEL NODE METASTASES

L Tafra , KM Verbanac, CJ Min
SM Purser, PC Ng, MS Swanson

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Sentinel node biopsy (SNB) is a minimally invasive diagnostic procedure that can determine the breast cancer patients (BCP) that may benefit from a complete axillary node dissection (AND). RT-PCR analysis of SNs offers the promise of improved staging sensitivity over routine histological methods. Purpose: For both these techniques to be applicable to the majority of BCP, a multi-center trial was undertaken to determine 1) the factors contributing to SNB failure and 2) the feasibility and clinical significance of RT-PCR to detect SN metastases. Method: An IRB-approved multi-center clinical trial (1995-1999) involving 41 surgeons enrolled BCP for SNB followed by a level I/II axillary node dissection. Peri-tumor injections of Tc99 and isosulfan blue dye were used. No investigator participated in a learning trial prior to participation. Alternate serial sections of each LN were designated for histology (H&E, immuno) or RT-PCR. PCR criteria included $\geq$2 PCR reactions from $\geq$2 cDNA preparations and stringent controls. PCR products were identified by ethidium bromide (EtBr) stain. To increase detection sensitivity, selected specimens were re-examined using 2X cDNA template and EtBr and/or by Southern blot (SoBt) analysis. We have previously identified mammaglobin and CEA as superior molecular markers for RT-PCR detection of occult breast disease. Results: The study compared 485 successfully mapped patients to 83 failed SNB (18 false negative and 65 with no SN identified). The analysis found no statistical difference between the two groups in tumor size, tumor location, type of gammma probe, vascular or lymphatic invasion, or method of diagnosis (tumor intact vs. prior surgery). The only significant differences were patient age (failure rate=5.1% age < 50 vs. 15.4% age $\geq$ 50, odds ratio = 3.46, 95% CI, 1.31-9.22) and prior surgical experience (failure rate=8.2% >10 cases vs. 17.9% $\leq$ 10 cases, odds ratio = 2.72, 95% CI, 1.30-5.71). Analysis was performed on 170 SNs from 92 BCP. Comparing mammaglobin RT-PCR analysis with histology results showed: H&Eneg/PCRneg = 58%; H&E +/PCR+ = 25%, H&Eneg/PCR+ = 14%, H&E+/PCRneg = 3%. By increasing the sensitivity (doubling cDNA template) 100% BCP H&E+ were PCR+ for mammaglobin and 50% of H&E neg were upstaged by PCR positivity. Conclusion: RT-PCR analysis for mammaglobin expression is a specific and sensitive method to detect breast SN metastases. Patient age and surgeon experience are the primary factors contributing to SNB failure and surgeons should not abandon complete AND until they obtain adequate experience.

The U.S. Army Medical Research and Materiel Command under DAMD17-98-1-8079

To Be Presented at the 23rd Annual San Sntonio Breast Cancer Symposium, December 6-9, 2000

RT-PCR detection of mammaglobin and carcinoembryonic antigen expression in breast cancer sentinel lymph nodes. *Verbanac KM, Min CJ, Lo K, Albrecht J, Purser SM, Swanson, MS, Bogey WM, and Tafra L. East Carolina University, Greenville NC; National Genetics Institute, Los Angeles CA; Anne Arundel Medical Center, Annapolis MD

Axillary staging is the most important factor in predicting breast cancer recurrence. Many patients are inaccurately staged by current methods: ~30% of pathologically node-negative women develop metastatic disease. Sentinel lymphadnectomy identifies the lymph nodes (LN) that directly drain a tumor and are most likely to harbor occult cells. Reverse transcriptase-polymerase chain reaction (RT-PCR) is a sensitive molecular technique that potentially detects one tumor cell among 10^6 normal cells. We have previously identified two specific markers for the RT-PCR detection of breast cancer LN metastases: mammaglobin (MAM) and carcinoembryonic antigen (CEA). Here we present results from RT-PCR analysis of 166 SLN from 92 patients enrolled in a multi-center study, 37% with histology-positive LN (histo; H&E±IHC) and 63% with histo- LN. As single markers, MAM and CEA PCR identified 94% and 84% of patients with histo+ LN, and 78% of these patients had LN that expressed both markers. Only one patient with histologically involved LN failed to express either marker. MAM was detected in 28% histo- patients. CEA expression in histo- nodes was 23%, upstaging 14 patients. In combination, five histo- patients expressed both markers and 19 histo- women were positive for either marker. Using PCR, staging would be affected in 44% of node-negative women. Marker expression correlates with tumor size and estrogen receptor negativity but not other clinico-pathologic indicators. Mean follow-up is 31 months; only seven women have recurred. Clinical monitoring for recurrence is ongoing to determine the significance of molecular staging. Mammaglobin and CEA are promising members of a multi-marker panel for RT-PCR detection of SLN micrometastases.

Presenting Author: Lorraine Tafra MD

Category: 1. Axillary/Sentinel Nodes

23rd Annual San Antonio Breast Cancer Symposium

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Effect of Technetium 99m Sulphur Colloid Injection Interval (TII) on Sentinel Node Biopsy (SNB). Arlene N Chua*, Donald L Lannin, Melvin S Swanson and Lorraine Tafra. Surgery, East Carolina University, Greenville, North Carolina; and The Breast Center, Anne Arundel Medical Center, Annapolis, Maryland.

Introduction:SNB alone without complete lymph node dissection is becoming the standard of care for breast cancer patients treated by experienced surgeons. The best time interval between radioisotope injection and SNB dissection (TII) has not been determined. It has been hypothesized that a short TII will decrease the chance of finding a sentinel node and a long TII will allow labeling of non-sentinel nodes. Objective: To determine the optimal TII for breast cancer SNB. Methods: An IRB approved multi-center clinical trial enrolled breast cancer patients between 1996 and 2000 for SNB using a peri-tumor injection of technetium 99m sulfur colloid and isosulfan blue dye. Eighteen academic and private practices participated and the TII was not restricted. The SN was defined as either blue only, both blue and hot or hot only. Results: Of 648 breast cancer patients enrolled, 544 had data to calculate the TII. The majority of patients had a complete lymph node dissection (562, 87%) and only this group was used to calculate the false negative rate. The false negative rate for the entire group was 11.9% , with 167 patients found to have metastatic disease in their axilla. The table summarizes the false negative and identification rates found in specific TII intervals. The TII >60<300 had a higher identification rate than <60 minutes (p=0.0021). The false negative rate tended to be higher for TIIs <20 and >300 minutes.
Conclusion: The optimal TII for breast SNB is probably between one and five hours. A larger group of false negatives would need to be analyzed to determine if timing influences the false negative rate.

TII (min.)	≤20	>20≤60	>60≤180	>180≤300	>300
N	80	69	178	151	66
Identification rate	83%	83%	92%	93%	85%
False negative rate	18%	6%	10%	9%	14%
Mean # SNs	1.89	1.92	2.28	2.45	2.34

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H Johnson*, T Jones, F Smith. John Wayne Cancer Institute, Santa Monica, CA, USA

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COMPARISON OF THREE METHODS FOR BREAST LYMPHATIC MAPPING

D Lannin*, R Cuenca, T Chadwell, M Iheanacho, L Tafra. East Carolina University, Greenville, NC & Anne Arundel Hospital, Annapolis, MD, USA

INTRODUCTION: Although intraparenchymal peri-tumor injection is considered the gold standard for breast lymphatic mapping, intradermal injection over the tumor and injection in Sappy's subareolar plexus have also been described. Our study compares all three methods in the same patients.

METHODS: Technetium 99 was injected intradermally over the tumor 30 minutes prior to surgery; 5 minutes before surgery Isosulfan blue dye or fluorescein dye was injected intraparenchymally around the tumor and the other dye was injected subcutaneously under the nipple in Sappy's plexus. A sentinel node was defined as any node that was hot, blue, or fluorescent.

RESULTS: At least one sentinel node was found in 62 out of 64 consecutive patients for an overall success rate of 97%. A total of 104 sentinel nodes were found; 94 (1.5 per patient) resulted from intradermal injection, 72 (1.1 per patient) from peri-tumor injection, and 82 (1.3 per patient) resulted from injection in Sappy's plexus. Location of injection significantly affected the number of nodes found ($P<.001$) but there was no difference between Isosulfan blue or fluorescein in either location. The majority of nodes (64 nodes, 63%) were hot, blue, and fluorescent. Of the 72 nodes detectable by peri-tumor injection, 66 (92%) were also hot from intradermal injection and 68 (94%) were also found by injection in Sappy's plexus. However, the latter techniques resulted in additional nodes that were not found by peri-tumor injection, 28 from intradermal injection and 14 from Sappy's plexus injection. Overall, 19 of 104 nodes (18%) were positive for metastasis. Of the 19 positive nodes, 14 resulted from peri-tumor injection, 16 from injection into Sappy's plexus, and 17 from intradermal injection suggesting that all three techniques identify a true biological sentinel node.

CONCLUSION: Injection anywhere within a quadrant of the breast usually drains to the same node or nodes. Intradermal injection or injection in Sappy's plexus seems to result in a higher chance of finding a sentinel node and less chance of false negatives. All three sites combined may yield greater sensitivity in sentinel node biopsy.

Abstract Deadline is August 28th 2000

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